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EFFECT OF MANURE APPLICATION ON INCIDENCE OF SOIL BORNE PLANT HUMAN PATHOGENS

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Period: February 1998 - June 2001

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Project participants:

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ABSTRACT

Two field sites near Humboldt - SK were treated yearly with hog and cattle manure at rates up to 960 kg N·ha^{-1} for four years from 1997 through 2000. The field sites were cropped to canola, spring wheat, barley and canola in 1997, 1998, 1999 and 2000, respectively. The soils were analyzed for microbial activity and microbial populations including fecal coliform bacteria, and total soil bacteria. Root rot and foliar disease was assessed in crop during growing seasons. Microbial activity in soils treated with cattle manure was higher than the soils injected with hog manure or urea treated-soils. Nitrous oxide emissions from soil increased with increasing rates of hog and cattle manure. For example, the highest N_2O emission rates were caused by addition of hog manure and cattle manure at 300 and 480 kg N·ha^{-1}, respectively. Nitrous oxide emission from cattle manure were on average two times lower that those corresponding to hog manure. Addition of cattle manure amended soil influenced the bacterial populations in the soil. On average, cattle manure treated soils exhibited 10-fold higher *Enterobacteriaceae* populations when compared to the remaining treatments. Several pathogens including *Rahnella*, *Serratia*, *Proteus*, *Leclercia*, and *Salmonella* species were identified in soils that received cattle manure whereas Pseudomonads were the dominant species identified in the hog manure-treated soil. Fecal coliforms were confirmed in soils which received hog or cattle manure. Increasing cattle manure rates from 120 to 480 kg N·ha^{-1}, increased fecal coliform population by *ca.* 20%. There was no indication that increased urea and/or hog manure rates increased fecal coliform populations in the soil. Application of hog or cattle manure did not cause increases in visual foliar disease, but increased root rot incidence in wheat in 1998. Leaf spot incidence was lower and leaves were bigger and more vigorous in plants from manure or urea treated soil. During the 1999 crop season, urea, hog or cattle manure did not cause increased foliar disease in barley. In fact, application of urea seemed to alleviate foliar disease of barley, and roots collected after harvest in the fall of 1999 did not show incidence of root rot related to manure additions to the soil. Similarly, in the summer of 2000 addition of urea, hog or cattle manure to the soil did not cause an increase in foliar disease in canola. Results from this study provide new information on microbial activity, populations and communities in manure amended soil. However, the link between presence of certain types of soil microorganisms to potential disease hazards is complex and cannot be derived or postulated simply from their incidence and activity in the soil.

A) EXECUTIVE SUMMARY

Soils from two field sites near Humboldt - SK were treated yearly with hog and cattle manure at rates up to 960 kg N·ha^{-1} from 1997 through 2000. The field site was cropped to canola, spring wheat, barley and canola in 1997, 1998, 1999 and 2000, respectively. Soils were sampled in the fall after harvest and in the spring, and soil microbial activity (CO_2 evolution), microbial populations (total heterotrophs, human pathogens and plant diseases) were assessed. Our results indicate that fresh application of hog and cattle manure at rates higher than 400 kg N·ha^{-1} increased microbial activity initially. However, CO_2 levels in hog manure decreased more rapidly than those corresponding to cattle manure treatments. This may be an indication that the hog manure is more readily used as a substrate for energy, nutrients and biomass production by the soil microbial population. Three manure sources (hog, cattle and poultry) were assessed for the presence of pathogenic bacteria. With the exception of *Klebsiellae*, presumptive *E. coli* bacteria were not detected in hog or cattle manure. However, a large population of these two bacteria was detected in fresh poultry manure. In fact, of the three manures assessed, hog manure had no *Escherichia coli* or *Klebsiellae* and had the lowest incidence of total *Enterobacteriaceae*. However, the populations of these pathogenic bacteria increased in soil receiving manure applications in both sites.

Addition of cattle manure influenced the diversity of soil bacteria, regardless of the rate of application. Pseudomonads were the dominant species identified in the hog manure-treated soil whereas several pathogens including *Rahnella*, *Serratia*, *Proteus*, *Leclercia*, and *Salmonella* species were identified in soils that received cattle manure. *Salmonella typhimurium* and *S. choleraesuis*, are pathogenic species identified that can cause typhoid fever and severe gastroenteritis in humans. Fecal coliforms were isolated and confirmed in soils that received hog or cattle manure but, fecal coliforms were confirmed only in the sample that received 75 kg N·ha^{-1} hog manure. However, there was no indication that increased hog manure rates also increased fecal coliform populations. No fecal coliforms were confirmed in soil receiving urea. In contrast, increasing cattle manure rates from 120 to 480 kg N·ha^{-1}, increased fecal coliform population by *ca.* 20%. Application of hog or cattle manure did not cause increases in visual foliar disease in wheat. Leaf spot incidence was lower and leaves were bigger and more vigorous in plants from manure or urea treated soil. However, application of hog and/or cattle manure to the soil increased root rot incidence in wheat.

In the third year, microbial activity in soils treated with cattle manure were on average at least two times higher than the soils injected with hog manure and three times higher than in the soils treated with urea. Similar to our previous observations, the lowest microbial activity was observed in the treatment receiving the high rate of urea. Nitrous oxide emission rates increased according to the rates of hog and cattle manure applied to the soil. For example, addition of hog

manure at 300 kg N·ha^{-1} produced the highest N_2O emission rate. Similarly, addition of cattle manure at 480 kg N·ha^{-1} also produced the highest N_2O emission rates, but these were on average two times lower that those corresponding to hog manure. During the Fall of 1999, addition of cattle manure appeared to cause an increase in the number of total heterotrophs, whereas the addition of urea and/or hog manure promoted little change in the total heterotroph populations. *Enterobacteriaceae* were found in all soils treated with cattle and hog manure, urea as well as the untreated controls. The cattle manure treated soils exhibited on average 10-fold higher *Enterobacteriaceae* populations when compared to the remaining treatments, thus it is reasonable to assume that cattle manure does contain slightly more *Enterobacteriaceae* as compared to hog manure. Additions of urea, hog and/or cattle manure affected the soil microbial community structure. For example, the FAME profiles of the whole soil communities associated with the high rates of hog and cattle manure were significantly different from the profiles of the communities associated with the control soils. The urea community was clearly separated from the communities associated with the hog and/or cattle manure treatments. However, no separation was observed using PCA amongst urea rates, thus suggesting that a less diverse microbial community composition was present in the urea-treated soil.

Addition of urea, hog or cattle manure to the soil did not cause increased foliar disease in barley during the 1999 crop season. In fact, application of urea seemed to alleviate foliar disease of barley. Increasing urea rates from 50 to 200 kg N·ha^{-1} decreased disease ratings. Similarly, barley roots collected after harvest in the fall 1999 did not show incidence of root rot related to manure additions to the soil. A plant disease-survey performed during the summer of 2000 on the canola crop indicated that addition of urea, hog or cattle manure to the soil did not cause visual foliar disease increase in canola during the 2000 crop season. However, all treatments exhibited traces to 1-5% of Aster yellows. Conversely, no traces of diseases caused by *Alternaria* and *Sclerotinia* species were detected.

During this research project, several human and plant pathogenic bacteria were isolated and identified. Some isolates are ubiquitous to soil and fresh water and are of unknown clinical significance. Some species are pathogenic bacteria known to invade human urinary tract and cause lesions on human skin. Other isolates occur in manures, soil and polluted water and are occasionally opportunistic pathogens to humans and animals. Results from our study provide new information on microbial activity, populations and communities in manure amended soil. However, the link between the presence of certain microorganisms in the soil and potential disease hazards is complex and cannot be derived or postulated simply from their incidence and activity in the soil.

B) TECHNICAL REPORT

BACKGROUND AND OBJECTIVES

The use of animal manure as a soil amendment is an important option for crop rotations and nutrient recycling as this system can contribute to better long term soil quality and fewer chemical inputs. In addition to controlling the release and availability of many important plant nutrients, the soil microbial community is the main source of soil - borne plant pathogens, thus it directly affects the sustainability and health of cropping systems. The successful application of manure to soil systems in the future will require an understanding of its influence on microbial characteristics of the soil. Hence, it is imperative to assess the inputs on the general soil microbial community and in particular the incidence of plant diseases and fecal coliforms (human enteropathogens), if any, as affected by manure applications in Saskatchewan.

The main objectives of this project were to investigate the impact of manure application on soil microbial community activity and composition, emphasizing plant and human pathogens. Emphasis was placed on those segments of the soil microbial population expected to impact human (enteropathogens) and plant (fungal) diseases.

PROJECT ACTIVITIES

In 1997, two field sites were established in the Black soil zone near Humboldt, Saskatchewan. The sites are located on a highly productive clay loam soil (Blaine Lake association) near Dixon and a marginal sandy loam (Meota association) near Burr, respectively. A replicated randomized complete block design was set up at each site. Treatments imposed included: (*i*) Control - no application, (*ii*) Hog manure, (*iii*) Cattle manure; and, (*iv*) Urea. Hog manure treatments were injected (wide × narrow row injection spacing) or surface applied in spring of 1997 before seeding and again in late fall after harvest. Cattle manure treatments were broadcast and incorporated or broadcast without incorporation in the spring of 1997 and again in late fall. Manure applications (kg of total $N \cdot ha^{-1}$) included hog (75, 150, 300) and cattle (120, 240, 480) at the Dixon site; and, hog (200, 400, 800) and cattle (225, 450, 900) at the Burr site. Urea was applied at 0, 50, 100 or 200 kg of available $N \cdot ha^{-1}$ at each site for comparison purposes. Both Dixon and Burr field sites were cropped to canola 1997, spring wheat in 1998, barley in 1999 and canola in 2000. Soil samples were taken from each plot by taking soil cores (5 cm diameter × 30 cm depth) in the fall of 1997 after harvest and again in spring of 1998 and fall 1998. In spring and summer of 1999, soil samples of 0-30 cm depth were taken from the Dixon site after manure additions made in the fall of 1998. The manure applications for 1999 included hog (0, 75, 150 and

300 kg of N·ha^{-1}) and cattle (applied at *ca.* 0, 120, 240 and 480 kg of total N·ha^{-1}). Again, urea was applied at 0, 50, 150 and 400 kg of N·ha^{-1} for comparison purposes. During the fall of 1999, soil samples of 0-30 cm depth were taken from the Dixon site prior to manure addition and in the spring of 2000 prior to seeding. Yearly manure applications included hog and cattle applied at *ca.* 100, 200, 400 kg of total N·ha^{-1}. Samples were brought to the laboratory and stored at 4°C until use. A total of 44 soil samples were obtained for each sampling time. Parameters assessed on the samples included: (*i*) microbial activity (CO_2 respiration and N_2O production), (*ii*) microbial populations; and, (*iii*) human and phyto-pathogenic microorganisms.

- **Microbial activity: Assessed by the CO_2 evolution method using gas chromatography**

Laboratory study: This study was included in order to provide important information on soil microbial activity as affected by fresh additions of manure. Soil samples, freshly treated with manure, were air dried, crushed using a wooden roller and sieved with a 2 mm sieve. A 200 g sample of soil was placed in air-tight 1.0L Mason jar equipped with a septum for gas sampling. The control, cattle and hog manure treatments were incubated in the dark at 20°C and at field capacity [*ca.* 27% (w·w^{-1})]. The cattle manure used in this study was air-dried, crushed and sieved using a 6-mm sieve. Total-N content of hog and cattle manure was 0.2 and 1.41%, respectively. The soil was amended with the solid cattle manure by shaking the soil with ground manure and water added to field capacity. Stock solutions of urea and liquid hog manure were prepared and added to the soil to obtain the required application rates of N at field capacity.

Field study: Soils sampled from field plots (both sites) receiving manure in 1997, 1998, 1999 and 2000 were collected. Fresh (60% MHC) soil samples (100 g) were incubated as described above, and microbial activity (mg C day·g^{-1}) assessed in samples (n=4) periodically up to 58 days.

CO_2 measurements: Headspace gas samples were taken from each of the 1.0L Mason jars periodically using a 1.0 cc syringe and CO_2 evolved was determined by gas chromatography. The lids of the Mason jars were opened after each sampling to allow for the re-oxygenation of the treatments and the weight of each jar was also taken at this time. Deionized water was added to the soil as required to ensure that the moisture content of the various treatments remained at field capacity throughout the duration of the incubation period.

• **Microbial populations**

Isolation and characterization of microbial community: Soils from the manure plots and hog, cattle and poultry manure were assessed for microbial populations using the agar plate count method (Bagley & Seidler, 1978; Foster & Rovira, 1975). Assessment of human pathogenic bacteria (*Enterobacteriaceae*, *Klebsiellae* and fecal coliforms) was performed in pure manure and soil samples from the two field locations using selective culture media. Fresh manure or soil samples were suspended in H_2O and then serially diluted (1/10). Aliquots of appropriate dilutions were spread plated onto the following culture media: (*i*) MacConkey's, (*ii*) MacConkey's plus carbenicillin to separate presumptive *Enterobacteriaceae* and non-*Enterobacteriaceae*; and, (*iii*) Trypticase soy agar for total heterotroph bacteria counts (Bagley & Seidler, 1978; Foster & Rovira, 1975). The Levine Eosin Broth Agar (EMB) medium was used to assess *E. coli* population in fresh manure. Inoculated plates (n=4) were incubated at 28°C and 37°C for *Enterobacteriaceae* and heterotrophs, respectively and bacterial colony-forming units (cfu) determined at 72 to 120 h of incubation. Fecal coliforms were assessed by the Most Probable Number (MPN) method. Fecal coliforms were used as indicator organisms for the presence of human enteropathogens (Franson *et al.*, 1989). A random number table was consulted and 50 colonies from a plate were isolated. Bacteria were streaked on original isolation medium, incubated for 48 h at 28°C, checked for purity and stored refrigerated for whole-cell fatty acid methyl ester (FAME) identification. Pathogenic bacteria were identified using the FAME clinical library profiles following the procedures described by MIDI (1990).

Identification of bacteria: Rhizobacteria were identified based on whole-cell cellular fatty acids, derivatized to methyl esters - FAMEs and analyzed by gas chromatography (GC), using the MIDI system (Microbial Identification System, Inc., Newark, USA). The procedures and protocols used for growing the cultures and instrument specifications are described by MIDI (1990). Bacterial isolates were grown on TSA plates at 28°C for 24 hs and bacterial cells (*ca.* 50 mg) collected. One ml of a methanolic NaOH (15% [wt·vol^{-1}] NaOH in 50% [vol·vol^{-1}] methanol) was added and cells were saponified at 100°C for 30 min. Esterification of fatty acids was performed with 2 ml of 3.25N HCl in 46% (vol·vol^{-1}) methanol at 80°C for 10 min. The FAMEs were extracted into 1.25 ml of 1:1 (vol·vol^{-1}) methyl-tert-butyl ether-hexane, and the organic extract washed with 3 ml of 1.2% (wt·vol^{-1}) NaOH before analysis by gas chromatography. FAME peaks were automatically integrated by a Hewlet-Packard integrator and bacterial isolates named using the MIDI Microbial Identification Software (Sherlock TSBA Library version 3.80; Microbial ID, Inc.). The FAME profile of *Xanthomonas maltophilia* ATCC 13637 was used as a reference for the MIDI determinations.

FAME profiles of the whole soil microbial communities: The FAME analysis of manure treated-soils was performed as described by Cavigelli *et al.*, (1995). Briefly, 5 g of soil was mixed with 5 ml of methanolic NaOH and then saponified at 100°C for 30 min. Further steps of this procedure have been described above. Soil FAME profiles were compared by principal component analysis (PCA) using a correlation matrix. Individual soil FAMEs were compared by ANOVA and homogeneity of variance tested.

- **Degree of plant disease infection (foliar and root rot) caused by soil phytopathogens**

A plant disease-survey was performed at the Dixon field site during the summer of 1998, 1999 and 2000 to assess disease incidence and severity. Infested plants and roots were brought to the laboratory and characterized for the presence of pathogenic fungi. Disease incidence and severity were evaluated on a 0–11 scale, while cereal root diseases were assessed by sampling 40 roots, washing and evaluating sub-crown internodes for root symptoms.

RESULTS AND DISCUSSION (YEAR I)

Microbial activity - CO_2 evolution

A. Soil freshly treated with manure - Laboratory study

- **Effect of fresh manure applications on short term soil microbial activity**

Application of fresh manure increased microbial activity with the highest level observed at 3 days after treating the soil with 400 kg $N \cdot ha^{-1}$ of cattle or hog manure (Fig. 1). However, the microbial activity in hog manure treated samples decreased more rapidly than in cattle manure amended soils, thus it may be an indication that the hog manure is more readily used as a substrate for energy, nutrients and biomass production by the soil microbial population. Urea fertilizer had relatively little impact on microbial activity (Fig. 1).

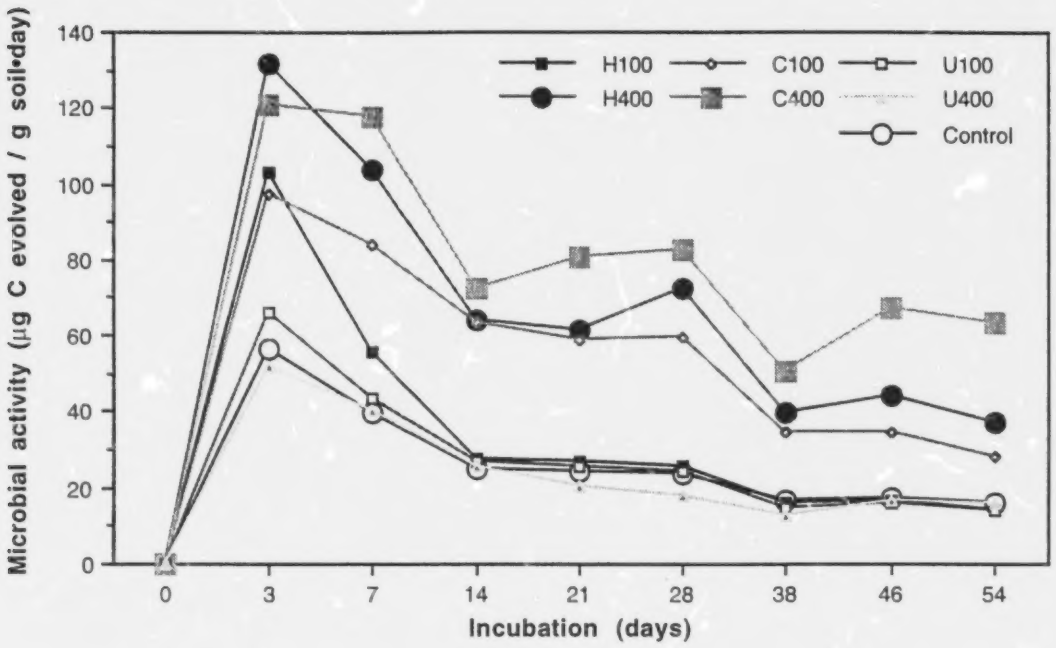

Figure 1. Effect of urea (U), cattle (C) and hog (H) manure application (50, 100, 400 kg N·ha^{-1}) on soil microbial activity (µg C per g of soil) in soil from the Dixon site. Data are an average of 4 replicates assessed at 3, 7, 14, 21, 28, 38, 46 and 54 days after application of manure.

B. Soils sampled from field plots (both sites) receiving manure in 1997

- **Effect of manure applications on soil microbial activity six months after initial application at the Dixon and Burr sites**

Application of urea, hog or cattle manure had no effect on measured microbial activity in the clay loam soil (Dixon site) sampled in the fall 6 months after initial manure application (Fig. 2). Conversely, on the sandy loam soil (Burr site) hog or cattle manure produced higher activity compared to the control or urea treated soils (Fig. 3). This is consistent with soils at the Burr site receiving higher rates of manure addition 6 months previously as compared to the Dixon site.

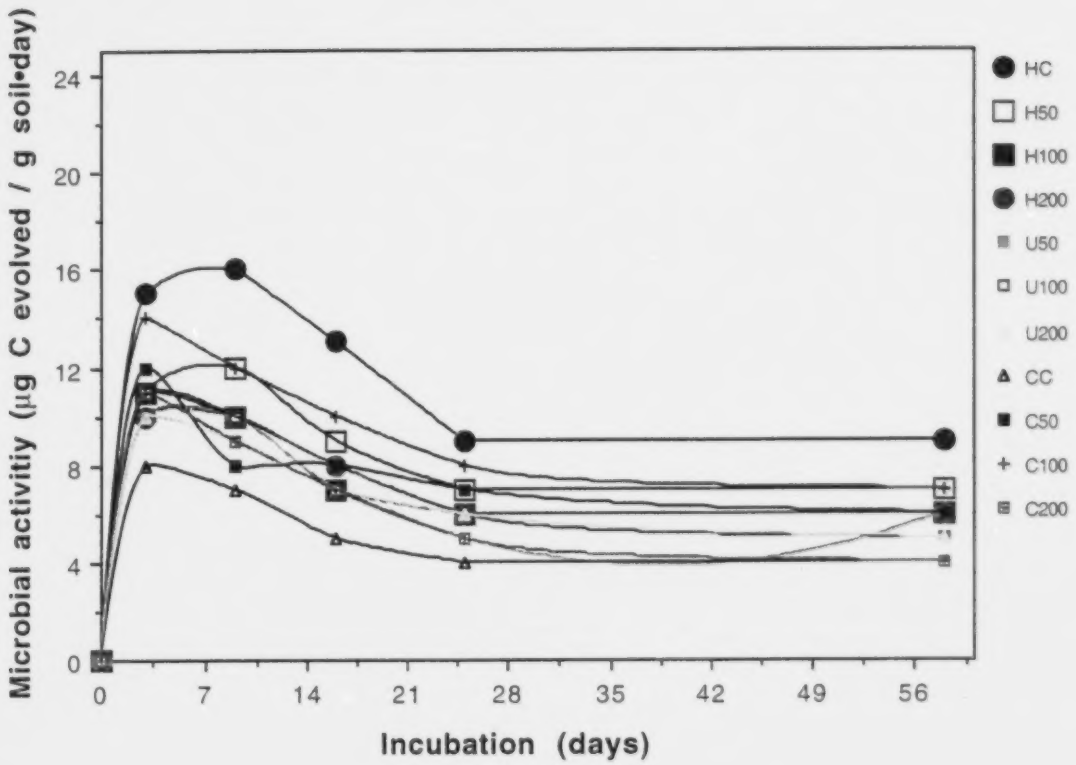

Figure 2. Soil microbial activity (µg C per g of soil) determined 6 months after field application of 50, 100 or 200 kg N·ha^{-1} urea (U), cattle (C) and/or hog (H) manure in soil from the Dixon site. Data are an average of 4 replicates incubated for 3, 9, 16, 25, and 58 days.

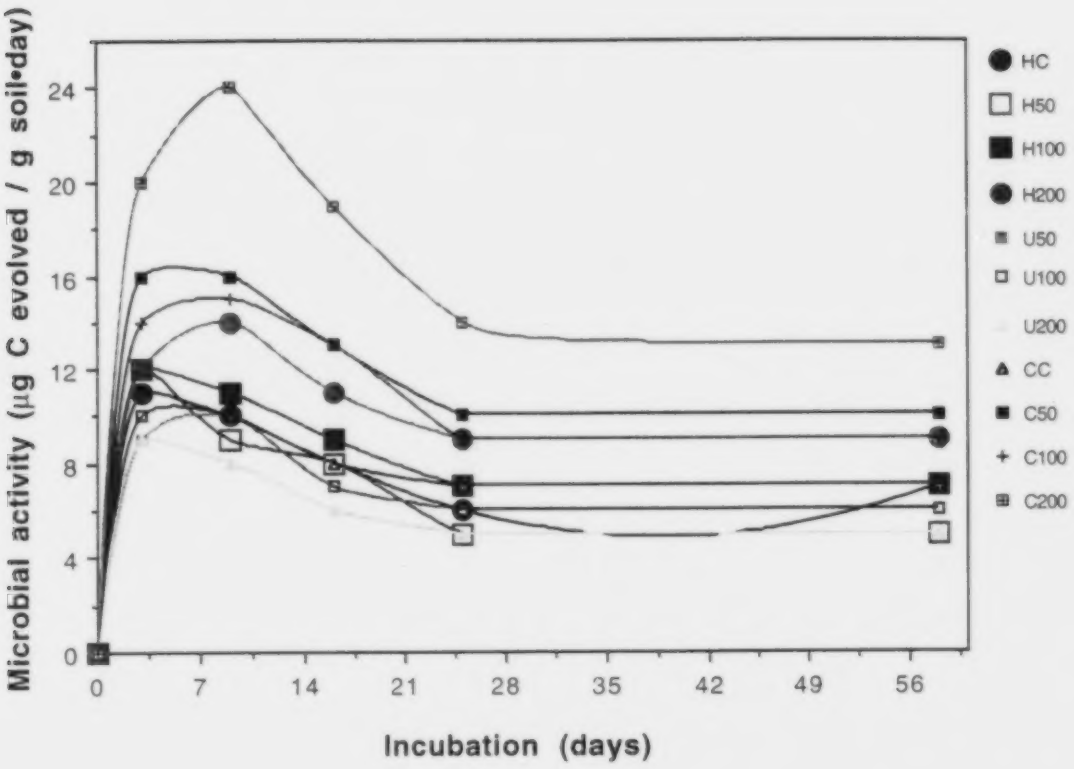

Figure 3. Soil microbial activity (µg C per g of soil) determined 6 months after field application of 50, 100 or 200 kg N·ha^{-1} urea (U), cattle (C) and/or hog (H) manure in soil from the Burr site. Data are an average of 4 replicates incubated for 3, 9, 16, 25, and 58 days.

- **Effect of fall manure applications on soil microbial activity in the following spring**

Application of cattle manure at rates higher than 240 kg N·ha^{-1} stimulated microbial activity in both sites. The highest activity was observed at 7 days incubation (Figs. 4 and 5). In contrast, application of urea produced the lowest increment.

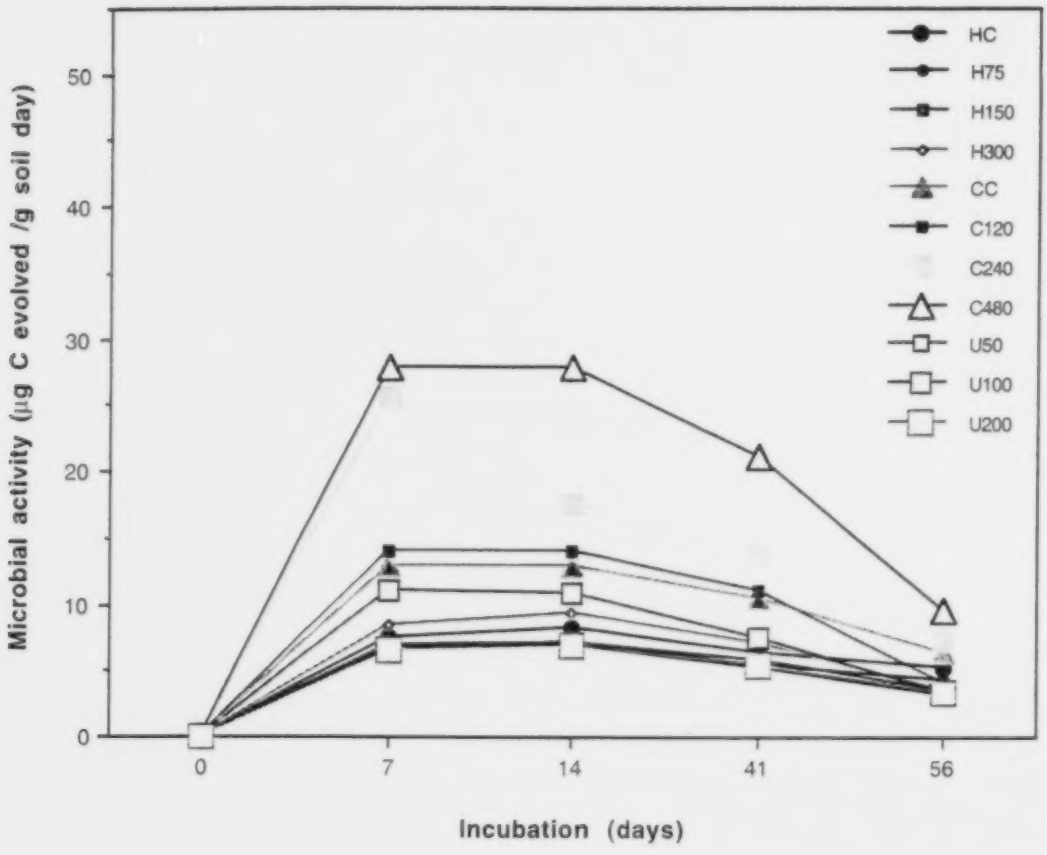

Figure 4. Soil microbial activity (µg C per g of soil) determined in the spring of 1998) after field application of 50, 100 or 200 kg N·ha^{-1} urea (U), cattle (C) and hog (H) manure during the previous fall in soil from the Dixon site. Data are an average of 4 replicates incubated for 7, 14, 41, and 56 days.

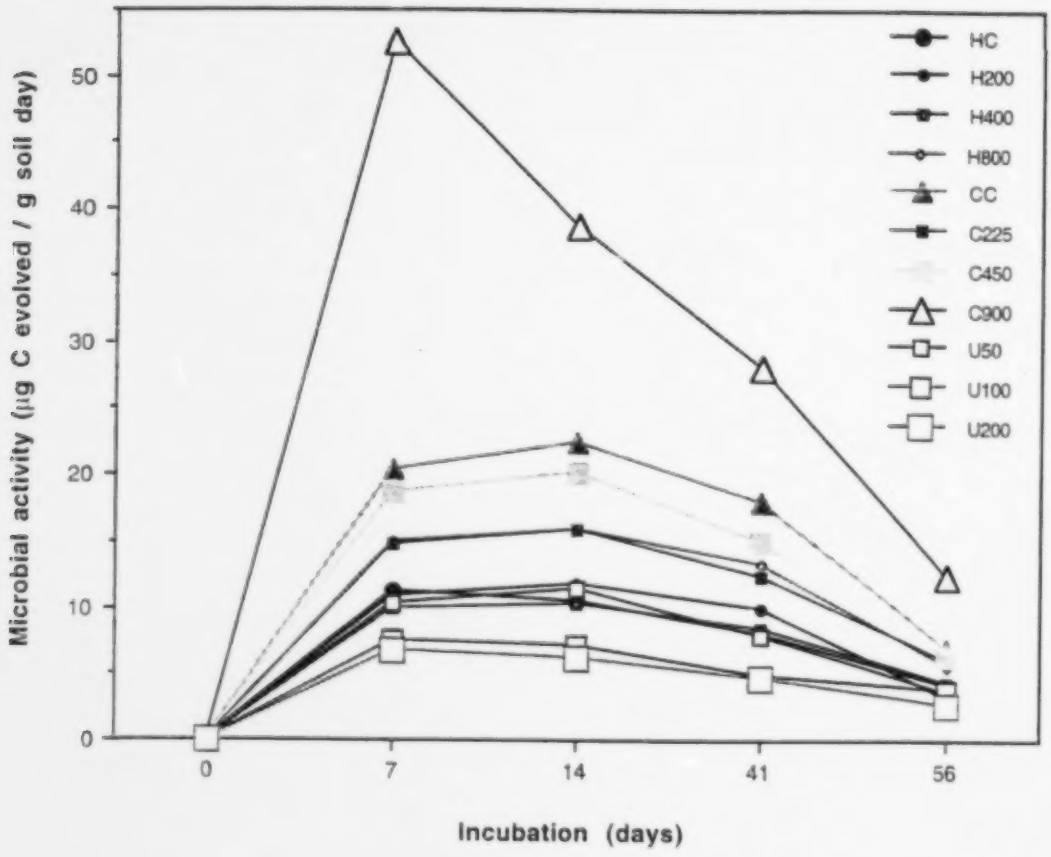

Figure 5. Soil microbial activity (µg C per g of soil) determined in the spring of 1998 after field application of 50, 100 or 200 kg N·ha^{-1} urea (U), cattle (C) and hog (H) manure in soil from the Burr site during the previous fall. Data are and average of 4 replicates incubated for 7, 14, 41, and 56 days.

Microbial populations

• Assessment of *E. coli* and/or presumptive *Klebsiellae* in fresh hog manure

Escherichia coli bacteria were not detected in hog or cattle manure. However, *Klebsiellae* were present in cattle manure. In contrast, fresh poultry manure sustained a high incidence *i.e.*, *ca.* 50% of these two pathogenic bacteria (Table 1). Of the three manure assessed, hog manure also had the lowest incidence of total *Enterobacteriaceae* (Table 1).

Table 1. Assessment of microbial populations present in hog, cattle and poultry fresh manure using selective culture media.

Microbial populations	Hog	Cattle	Poultry
	.....	(cfu·g^{-1}*)	.....
Escherichia coli	ND§	ND	1.5×10^3
Presumptive *Klebsiellae*	ND	2.1×10^2	1.6×10^2
Total *Enterobacteriaceae*	4.5×10^2	6.4×10^4	2.7×10^5
Total heterotrophs	3.5×10^5	1.1×10^7	1.5×10^8

*Fresh weight basis; §Not detected.

- **Effect of manure applications on the culturable *Enterobacteriaceae* population in the soil at the Dixon site**

At the Dixon site, total heterotrophs, *Enterobacteriaceae* and presumptive *Klebsiellae* populations increased slightly with increasing rates of hog manure. Conversely, when cattle manure was applied, *Enterobacteriaceae* and presumptive *Klebsiellae* populations increased *ca.* ten fold as compared to the untreated control (Table 2).

Table 2. Culturable bacteria populations (cfu·g^{-1}) present in soil collected at the Dixon site during the Fall 97.

Treatment	Nitrogen (kg·ha^{-1})	Total heterotrophs (cfu·g^{-1} soil) (×10^8)	*Enterobacteriaceae* (cfu·g^{-1} soil) (×10^2)	Incidence (cpm)*	Presumptive *Klebsiellae* (cfu·g^{-1} soil) (×10^2)	Incidence (cpm)
Control	0	5	41	8.4	24	4.9
Hog	75	6	3	0.5	6	1.0
Hog	150	60	120	2.0	28	0.5
Hog	300	35	31	0.9	110	3.1
Control	0	120	390	3.2	34	0.3
Cattle	120	49	140	2.8	27	0.6
Cattle	240	49	3300	67.6	150	3.0
Cattle	480	140	5800	40.1	310	2.1
Urea	50	170	250	1.5	400	2.3
Urea	100	150	240	1.6	210	1.4
Urea	200	95	680	7.2	330	3.5

* Incidence (cpm): Counts per million of total heterotroph population.

- **Effect of hog or cattle manure applications on the *Enterobacteriaceae* population in the soil at the Burr site**

Similar to the Dixon site, *Enterobacteriaceae* and presumptive *Klebsiellae* populations increased slightly with manure applications at Burr, but total counts were not higher than those obtained for the urea treatments (Table 3). Cattle manure exerted a sixty fold increase in the *Enterobacteriaceae* and presumptive *Klebsiellae* populations as compared to the untreated control (Table 3).

Table 3. Culturable bacteria populations (cfu·g^{-1}) present in soil collected at the Burr site during the fall of 1997.

Treatment	Nitrogen (kg·ha^{-1})	Total heterotrophs (cfu·g^{-1} soil) (×10^9)	*Enterobacteriaceae* (cfu·g^{-1} soil) (×10^4)	Incidence (cpm)*	Presumptive *Klebsiellae* (cfu·g^{-1} soil) (×10^4)	Incidence (cpm)
Control	0	2	2	10.3	11	56.7
Hog	200	17	24	14.1	320	188.9
Hog	400	5	0.1	0.1	6	11.2
Hog	800	5	51	109.1	11	24.5
Control	0	24	32	13.4	19	7.9
Cattle	225	17	1200	694.2	1500	887.2
Cattle	450	15	22	14.4	26	16.9
Cattle	900	18	220	117.8	72	39.4
Urea	50	2	25	113.6	34	153.2
Urea	100	9	10	11.7	2	1.9
Urea	200	7	2	3.1	8	11.6

* Incidence (cpm): Counts per million of total heterotroph population.

- **Effect of hog or cattle manure applications on the incidence of fecal coliforms (entero-pathogens) population in the soil**

Fecal coliforms were isolated and confirmed in soils which received hog or cattle manure. However, there was no indication that increased manure rates also increased fecal coliform populations. Fecal coliforms increased slightly with manure applications, but total counts were not higher than those obtained for the urea treatments (Table 4). No fecal coliforms were found in soil receiving urea (Table 4).

Table 4. Presumptive and confirmatory tests of fecal coliforms in soil from the Dixon site sampled in the fall of 1998.

Treatment	Nitrogen ($kg \cdot ha^{-1}$)	Most Probable Number	
		Presumptive ($\times 10^3$)	Confirmatory ($\times 10^3$)
		..... (counts per 100 ml)	
Control	0	1.0	ND*
Hog	75	5.5	1.1
Hog	150	2.6	1.0
Hog	300	2.6	1.2
Control	0	1.5	ND
Cattle	120	2.3	0.2
Cattle	240	8.0	0.4
Cattle	480	8.0	1.0
Urea	50	0.2	ND
Urea	100	ND	ND
Urea	200	0.3	ND
Hog manure		11.0	2.7

*Not detected.

- **Effect of hog or cattle manure applications on wheat disease during the first year**

Application of hog or cattle manure did not increase foliar leaf disease in wheat. In fact, our results suggest that hog manure and urea application reduced foliar diseases. However, application of cattle manure did not reduce severity of foliar diseases (Table 5).

- **Effect of hog or cattle manure applications on plant root rot disease (fungi) during the first year**

Application of hog and cattle manure appeared to increase the incidence of root rot by 70-95%, and 67-75%, respectively. Results were compared to the hog and cattle controls which had 57.5% and 62% root rot incidence, respectively (Table 5).

Table 5. Foliar and common root rot disease assessment in wheat during the summer of 1998. Plants were grown on canola stubble in plots previously treated with hog manure in the fall of 1997 at the Dixon site. Data are average of 4 replications.

Treatment	Rate (kg N·ha^{-1})	Total leaf spot* (%)	Disease rating** (%)	Disease incidence** (%)
Control	0	10	15.8	57.5
Hog	75	9.1	32.0	85.0
Hog	150	7	21.3	70.0
Hog	300	6.2	41.3	95.0
Control	0	10	23.0	62.5
Cattle	120	10	24.8	67.5
Cattle	240	9.5	32.8	75.0
Cattle	480	8.5	25.8	70.0
Urea	50	9	20.8	77.5
Urea	100	7	24.0	80.0
Urea	200	5.7	23.5	75.0

*Foliar disease rating based on a 0 – 11 scale (0: no disease; 11: most severe). Sampled at the milk-soft dough stage; **Common root rot on sub-crown internodes.

RESULTS AND DISCUSSION (YEAR II)

Soil Microbial Activity

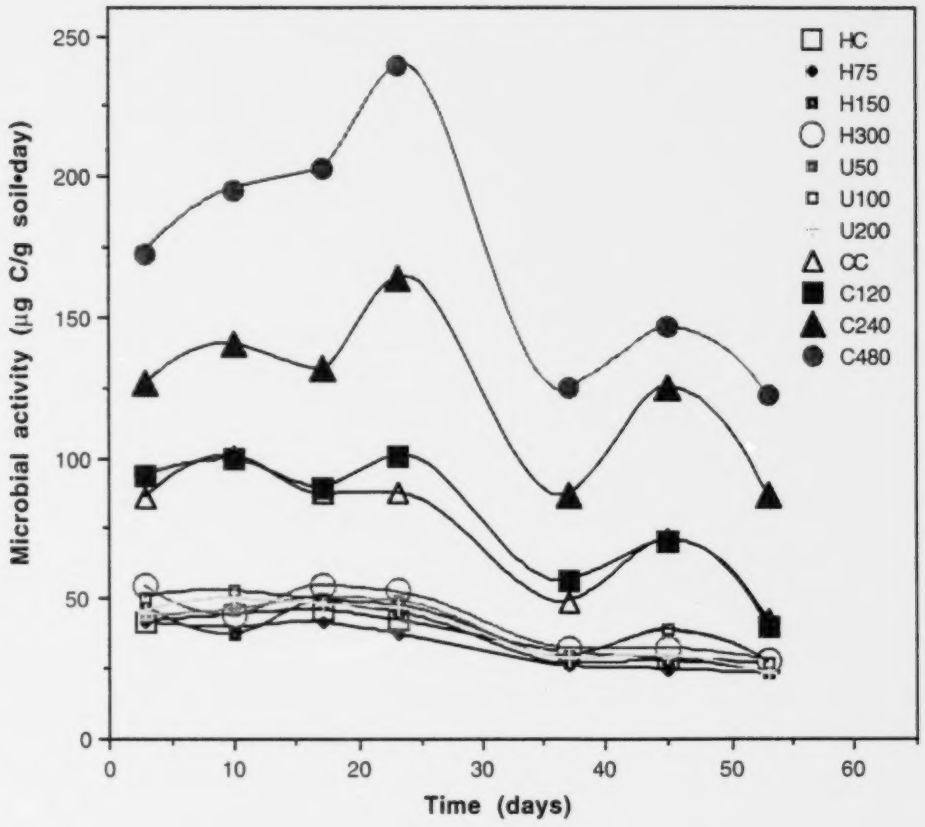

Figure 2. Effect of urea (U), hog (H) and cattle (C) manure applications (kg $N \cdot ha^{-1}$) made in the fall of 1998 on microbial activity (µg $C \cdot g^{-1}$ of soil) in soil from the Dixon site during the spring of 1999. Data are an average of 4 replicates assessed.

The microbial activity, assessed by CO_2 evolution, in soil samples collected in the spring of 1999 after three previous manure additions indicated that applications of urea, hog and/or cattle manure to the soil were beginning to affect the microbial activity differently. For example, during the 53-day soil incubation study, the microbial activity of soils treated with cattle manure was on average 4-fold higher than the soils injected with hog manure or urea. The higher microbial

activity was observed for the treatments receiving 480 and 240 kg N·ha^{-1} at 17 and 23 days in the cattle manure treated soils, respectively (Fig. 2). Hog manure and urea produced no effects on microbial activity during the spring, except that the highest activity was observed at the beginning of the study *i.e.*, at 3 and 10 days of incubation. However, the CO_2 levels tended to decrease towards the end of the study (Fig. 2). These results confirm our previous observations that hog manure was more readily used as an energy source for microbial biomass and activity than that of cattle manure, and that repeated applications of cattle manure results in a more sustained elevation of microbial activity than hog manure.

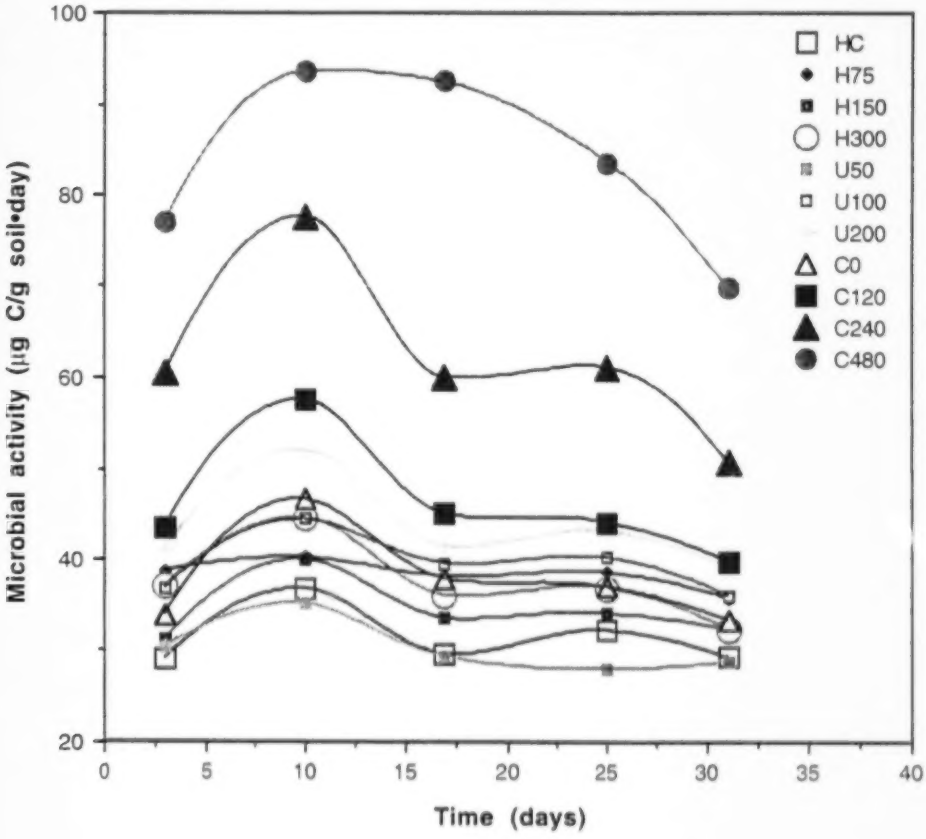

Figure 7. Effect of urea (U), hog (H) and cattle (C) manure application (kg N·ha^{-1}) on soil microbial activity (µg C·g^{-1} of soil) in soil samples collected from the Dixon site at mid growing season during the summer of 1999. Data are an average of 4 replicates assessed.

Soil samples obtained again during the summer of 1999 were also assessed for microbial activity. Applications of urea, hog and/or cattle manure to the soil during the fall of 1998 affected microbial activity, however, CO_2 evolution on these soil samples were much lower (*ca.* 50% lower) as compared to those obtained 3 months earlier during the spring (Fig. 7). The CO_2 release of soils treated with cattle manure was between *ca.* 60 and 95 $\mu g\ C \cdot g^{-1}$ soil. These figures are on average 2-fold higher than the soils injected with hog manure or urea. However, the application of urea at 200 kg $N \cdot ha^{-1}$ produced the highest microbial activity when compared to the treatments corresponding to hog manure (Fig. 7).

Microbial populations

• Fall - 1998 Bacterial identifications

Total soil bacteria isolated from samples taken in the fall of 1998 from the Dixon site were identified using fatty acid methyl ester profiles (MIDI). Potential human pathogenic bacteria were isolated using: (*i*) MacConkey's Agar for total *Enterobacteriaceae*; (*ii*) MacConkey's Base with Inositol and Carbenicillin for presumptive *Klebsiellae*. Approximately 10% of the average number (selected by a random number table) of isolates in each treatment were identified based on bacterial fatty acid using the clinical MIDI library for the identification of clinical bacteria.

Table 6. Microbial identification of bacterial strains associated with soils receiving cattle manure in the fall of 1998 and collected during the fall of 1998 immediately after the manure application at the Dixon experimental site.

Sample	Manure rate (kg N/ha)	MIDI - Identification	Similarity Index
1754	120	No Match	–
1755	120	No Match	–
1756	120	No Match	–
1759	120	No Match	–
1743	120	*Bordetella avium*	0.423
1758	120	No Match	–
1768	120	No Match	–
1757	120	No Match	–
1740	120	*Bordetella bronchiseptica*	0.605
1751	120	*Bordetella bronchiseptica*	0.398
11750	240	*Brucella melitensis*	0.307
11748	240	*Escherichia coli* subgroup B	0.801
11745	240	*Brucella melitensis*	0.583
11761	240	*Escherichia coli* subgroup E	0.040
11763	240	*Escherichia coli* subgroup B	0.430
11762	240	No Match	–
11753	240	*Brucella melitensis*	0.688
11737	240	*Ochrobactrum anthropi*	0.167
11767	240	No Match	–
11733	240	*Escherichia coli* subgroup A	0.561
11744	240	*Brucella melitensis*	0.363
11747	240	*Brucella melitensis*	0.335
11749	480	*Brucella melitensis*	0.410
11738	480	*Escherichia coli* subgroup A	0.754
11734	480	*Salmonella choleraesuis*	0.751
11739	480	*Ochrobactrum anthropi*	0.233
11735	480	*Klebsiella oxytoca*	0.720
11736	480	*Klebsiella oxytoca*	0.691
11764	480	*Brucella melitensis*	0.560
11765	480	No Match	–
11752	480	*Brucella melitensis*	0.228
11760	480	*Pseudomonas stutzeri*	0.333
11766	480	*Bordetella avium*	0.580
11746	480	*Ochrobactrum anthropi*	0.198

A total of 122 bacteria isolated from the Dixon site during the fall 1998 and summer 1999 were identified (Tables 6, 8). In samples collected in the fall 1998, no potential human pathogenic bacteria could be isolated from the control plots and plots receiving urea and/or hog manure immediately after manure applications during the fall (data not shown). However, the FAME-MIDI analysis identified at least four potential human pathogenic bacteria including: *Bordetella*

bronchiseptica, *Brucella melitensis*, *Escherichia coli*, *Klebsiella oxytoca* and *Salmonella choleraesuis*, followed application of cattle manure (Table 6). Increased rates of cattle manure resulted in an increased diversity of potential human pathogenic bacteria present in the soil. For example, only two bacterial species *i.e.*, *Bordetella avium* and *B. bronchiseptica* were detected in soils that received cattle manure at 120 kg N·ha^{-1}. In contrast, three and seven different species of pathogenic bacteria including *Bordetella*, *Brucella*, *Klebsiella*, *Ochrobactrum*, *Pseudomonas* and *Salmonella* species were identified by the MIDI library in soil samples that received cattle manure at 240 and 480 kg N·ha^{-1}, respectively (Table 6).

• 1999 - Microbial Populations

Table 7. Assessment of total bacterial populations (colony forming units - cfu) in soil samples collected at the Dixon experimental site during the summer of 1999.

Treatment	Rate (kg N·ha^{-1})	*Enterobacteriaceae* (× 10^3)	Presumptive *Klebsiellae* (× 10^4)	Total heterotrophs (× 10^8)
		(cfu per g dry soil)		
Hog	0	3.9	1.6	7.4
Hog	75	15.0	5.9	6.0
Hog	150	62.0	2.0	6.4
Hog	300	46.0	0.9	4.5
Urea	50	3.4	0.9	4.7
Urea	100	5.2	1.0	9.7
Urea	200	120.0	1.6	6.1
Cattle	0	17.0	1.3	6.2
Cattle	120	13.0	1.3	9.0
Cattle	240	65.0	7.5	11.0
Cattle	480	26.0	2.2	11.0

Assessment of total bacterial populations in soil samples collected at the Dixon experimental site indicate that addition of urea, hog and/or cattle manure at lower rate applications did not change the number of total heterotrophs in the soil samples during the summer 1999. A slightly higher number of total heterotrophs were detected in soil samples that received cattle manure at 240 and 480 kg N·ha^{-1} when compared to the remaining treatments (Table 7). This observation may be related to sampling time with regards to timing of manure application relative to sampling. That is,

manure applications were done during the fall of 1998 and samples collected during the summer of 1999. Similarly, results presented in Table 7 also show that *Enterobacteriaceae* and presumptive *Klebsiellae* were found in soils treated with hog manure, urea and the untreated controls.

• Summer - 1999 Bacterial identifications

Table 8. Microbial identification from bacterial isolates collected at the Dixon experimental site during the summer of 1999.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
14016	Hog	0	*Pseudomonas putida*	0.852
14015	Hog	0	*Pseudomonas putida*	0.849
13705	Hog	0	No Match	–
14014	Hog	0	*Pseudomonas putida*	0.832
14013	Hog	0	*Pseudomonas fluorescens*	0.906
14017	Hog	0	*Pseudomonas fluorescens*	0.852
14018	Hog	0	*Pseudomonas putida*	0.867
14019	Hog	0	*Pseudomonas putida*	0.839
14020	Hog	0	*Pseudomonas putida*	0.827
14021	Hog	0	*Pseudomonas fluorescens*	0.884
14022	Hog	75	*Pseudomonas fluorescens*	0.929
14023	Hog	75	*Pseudomonas fluorescens*	0.627
14024	Hog	75	*Pseudomonas fluorescens*	0.912
14025	Hog	75	*Pseudomonas fluorescens*	0.696
13709	Hog	75	*Rahnella aquatilis*	0.758
14026	Hog	75	*Pseudomonas fluorescens*	0.982
13710	Hog	75	*Yersinia intermedia*	0.241
14027	Hog	75	*Pseudomonas fluorescens*	0.815
14029	Hog	150	*Pseudomonas fluorescens*	0.849
13727	Hog	150	*Pseudomonas putida*	0.774
14028	Hog	150	*Pseudomonas putida*	0.830
14030	Hog	150	*Pseudomonas putida*	0.820
14031	Hog	150	*Pseudomonas putida*	0.797
14032	Hog	150	*Pseudomonas putida*	0.829
14033	Hog	150	*Pseudomonas viridiflava*	0.642
14034	Hog	150	*Pseudomonas putida*	0.859
14035	Hog	150	*Pseudomonas putida*	0.923
13697	Hog	150	*Pseudomonas stutzeri*	0.780
14036	Hog	150	*Pseudomonas putida*	0.860
13698	Hog	300	*Rahnella aquatilis*	0.578
13699	Hog	300	*Rahnella aquatilis*	0.586
13700	Hog	300	*Serratia liquefaciens*	0.455

Table 8. (cont'd) Microbial identification from bacterial isolates collected at the Dixon experimental site during the summer of 1999.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
13702	Hog	300	*Acinetobacter baumannii*	0.120
14037	Hog	300	*Pseudomonas putida*	0.744
13703	Hog	300	*Serratia marcescens*	0.399
13704	Hog	300	*Rahnella aquatilis*	0.618
14038	Hog	300	*Pseudomonas stutzeri*	0.623
14039	Hog	300	*Pseudomonas viridiflava*	0.844
13711	Urea	50	*Pseudomonas fluorescens*	0.845
13712	Urea	50	*Pseudomonas putida*	0.779
13713	Urea	50	*Pseudomonas fluorescens*	0.899
13714	Urea	50	*Yersinia enterocolitica*	0.545
13715	Urea	50	*Pseudomonas putida*	0.863
13716	Urea	50	*Pseudomonas fluorescens*	0.879
13717	Urea	50	*Pseudomonas fluorescens*	0.891
13718	Urea	50	*Pseudomonas putida*	0.833
13719	Urea	50	*Rahnella aquatilis*	0.689
13720	Urea	50	*Pseudomonas putida*	0.834
13721	Urea	100	*Acinetobacter johnsonii*	0.150
13722	Urea	100	*Pseudomonas fluorescens*	0.774
13723	Urea	100	*Rahnella aquatilis*	0.714
13724	Urea	100	*Rahnella aquatilis*	0.635
13725	Urea	100	*Rahnella aquatilis*	0.704
13730	Urea	200	*Flavimonas oryzihabitans*	0.724
13729	Urea	200	*Acinetobacter johnsonii*	0.170
13728	Urea	200	*Acinetobacter calcoaceticus*	0.351
13731	Urea	200	*Acinetobacter johnsonii*	0.138
13732	Urea	200	*Acinetobacter johnsonii*	0.109
13733	Urea	200	*Pseudomonas viridiflava*	0.777
13734	Urea	200	*Rahnella aquatilis*	0.642
13735	Urea	200	*Rahnella aquatilis*	0.735
13736	Urea	200	*Acinetobacter johnsonii*	0.214
13740	Urea	200	*Acinetobacter baumannii*	0.338
13739	Cattle	0	*Pseudomonas putida*	0.689
14041	Cattle	0	*Pseudomonas fluorescens*	0.795
14042	Cattle	0	*Pseudomonas putida*	0.772
14043	Cattle	0	No Match	–
14045	Cattle	0	*Pseudomonas putida*	0.793
14046	Cattle	0	*Pseudomonas putida*	0.896
13998	Cattle	120	*Rahnella aquatilis*	0.648
13999	Cattle	120	*Pseudomonas putida*	0.358

Table 8 (cont'd). Microbial identification of bacterial strains isolated at the Dixon experimental site during the summer of 1999.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
14000	Cattle	120	*Serratia marcescens*	0.338
14001	Cattle	120	*Proteus vulgaris*	0.488
14002	Cattle	120	*Proteus vulgaris*	0.433
14003	Cattle	120	No Match	–
14004	Cattle	120	*Salmonella typhimurium*	0.374
14005	Cattle	240	*Salmonella choleeraesuis*	0.424
14006	Cattle	240	*Salmonella typhimurium*	0.425
14007	Cattle	240	*Leclercia adecarboxylata*	0.359
14008	Cattle	240	*Leclercia adecarboxylata*	0.428
14009	Cattle	240	*Salmonella typhimurium*	0.426
14010	Cattle	240	*Serratia liquefaciens*	0.488
14011	Cattle	240	*Leclercia adecarboxylata*	0.307
14012	Cattle	240	No Match	–
14040	Cattle	240	*Serratia liquefaciens*	0.441
13726	Cattle	480	*Pseudomonas putida*	0.517
13741	Cattle	480	*Pseudomonas stutzeri*	0.328
13742	Cattle	480	No Match	–

Utilization of the MIDI microbial identification system using bacterial fatty acids coupled with the MIDI library for the identification of clinical bacteria indicated that Pseudomonads made up the greatest number of soil bacteria identified in soils treated with hog manure. For example, of the 38 isolates identified, 29 isolates were *Pseudomonas* spp. In fact, Pseudomonads constituted *ca.* 90% of all bacteria present in soils that received hog manure at rates up to 150 kg N·ha^{-1} (Table 8). Pseudomonads are ubiquitous to soils, thus it is normal to have a high number of this species in a soil sample. However, three additional bacterial species including *Acinetobacter*, *Rahnella*, and *Serratia* spp were identified in soil samples treated with hog manure at 150 kg N·ha^{-1}. However, *Acinetobacter*, *Rahnella*, and *Serratia* species occur naturally in soil, water and sewage and are classified as opportunistic human pathogens and/or of unknown clinical significance. Similarly, these bacterial species were also found in soils that received urea applications, but there were no particular trends related to a bacterial species and/or species abundance in each of the urea treatments (Table 8). Unidentified isolates which have no match in the MIDI library are unknown bacterial species which have no profiles available in the MIDI library.

These results confirm our observations from the previous two years which suggest that addition of hog manure up to 150 kg N·ha^{-1} to the soil did not cause an increase in the incidence of

a particular bacterial species including potential pathogenic bacteria (Table 8). Our results also demonstrate that additions of cattle manure influenced the diversity of bacteria isolates from the soil, regardless of the rate of application. Whereas Pseudomonads were the dominant species identified in the hog manure-treated soil samples, the same does not hold true when cattle manure was surface applied to the soils (Table 8). For example, we identified Pseudomonads (n=9 isolates), *Rahnella aquatilis* (n=1), unknown clinical significance and ubiquitous to soil and fresh water; *Serratia marcescens* (n=3), occasionally an opportunistic human pathogen; *Proteus vulgaris* (n=2), occurs in manures, soil and polluted water. *Proteus* species are pathogenic bacteria known to invade human urinary tract and cause lesions on human body skin; *Leclercia adecarboxylata* (n=3), unknown clinical significance; *Salmonella typhimurium* (n=2) and *Salmonella choleraesuis* (n=1). These two species are pathogens to humans and animals. They are responsible for causing typhoid fever and gastroenteritis in humans. It is important to note that the presence of a pathogen in the soil system does not directly translate into a conclusive human health hazard since transmission from soil to humans will depend on host factors.

- **Incidence of soil entero-pathogens (fecal coliforms)**

Application of urea, hog or cattle manure influenced the fecal coliform (entero-pathogens) populations in the soils. For example, fecal coliforms were isolated and confirmed in soils which received hog or cattle manure. However, fecal coliforms were confirmed in only one sample corresponding to hog manure applications *i.e.*, at 75 kg $N \cdot ha^{-1}$. There was no indication that increased hog manure rates also increased fecal coliform populations. Fecal coliforms increased slightly with cattle manure applications *e.g.*, application of 240 and 480 kg $N \cdot ha^{-1}$ caused an increase in presumptive fecal coliform numbers. These results were also confirmed by the MPN test (Table 9). No fecal coliforms were confirmed in soil receiving urea (Table 9).

- **Foliar disease assessment**

Results from the foliar disease incidence assessed in barley during the summer of 1999 is presented in Table 10. Our results indicate that addition of urea, hog or cattle manure to the soil were not associated with foliar disease in barley during the 1999 crop season. This can be confirmed by the fact that spot blotch and/or net blotch were found on all treatments including the untreated controls or urea treatments. In fact, application of urea seemed to alleviate foliar disease of barley as increasing urea rates from 50 to 400 kg $N \cdot ha^{-1}$ decreased disease ratings (Table 10). We also detected trace amounts of loose smut, which have been indicated as percentages. For example, with the exception of the treatment that received 150 kg $N \cdot ha^{-1}$, loose smut was not

found in plants grown in soils that received other treatments of hog manure (Table 10). All remaining treatments contained only traces of loose smut.

Table 9. Presumptive and confirmatory tests (most probable number - MPN) of fecal coliforms present in soil samples collected at the Dixon site during the summer of 1999.

Treatment	Rate (kg N·ha^{-1})	Presumptive (MPN × 10^3 per 100 ml or 10 g fresh soil)	Confirmatory
Hog	0	11.9	ND*
Hog	75	2.3	2.5
Hog	150	1.6	ND
Hog	300	8.7	ND
Urea	50	ND	ND
Urea	100	1.2	ND
Urea	200	3.0	ND
Cattle	0	2.7	ND
Cattle	120	1.7	ND
Cattle	240	1.5	0.1
Cattle	480	7.4	148.5

*Not detected.

Table 10. Foliar disease (leaf spots and loose smut) of barley assessed at the Dixon experimental site during the summer of 1999. Data are average of 4 replications.

Treatment	Rate (kg N·ha^{-1})	Leaf Spots present	Disease Rating* Ave 0-11	Growth Stage majority	loose smut trace (%)
Hog	0	spot & net	4.50	milk	ND†
Hog	75	spot & net	4.75	soft dough	ND
Hog	150	spot & net	4.25	soft dough	50
Hog	300	spot & net	3.50	soft dough	ND
Urea	50	spot & net	4.75	soft dough & milk	ND
Urea	100	spot & net	4.00	soft dough	25
Urea	200	spot & net	3.25	soft dough	50
Cattle	0	spot & net	4.50	soft dough	50
Cattle	120	spot & net	4.75	soft dough	75
Cattle	240	spot & net	4.50	soft dough	25
Cattle	480	spot & net	3.75	soft dough	ND

*Foliar disease rating based on a 0 – 11 scale (0: no disease; 11: most severe); †Not detected.

• Root rot disease assessment

Barley roots collected after harvest in the fall of 1999 were assessed for root disease incidence. Our data (Table 11) suggest that manure applications did not cause significant increases in plant root rot disease (fungi) in the barley crop as was observed for wheat in the previous year. However, the hog manure plots, as a whole, increased slightly in average root rot incidence by 3% when compared to the control plots. Application of urea decreased root rot incidence as did the cattle manure-applied plots.

Table 11. Incidence of root disease (common root rot) of Barley cultivated at the Dixon experimental site during the Summer 1999.

Sample	Rate ($kg\ N \cdot ha^{-1}$)	Disease Rating (%)	Disease Incidence (%)
Hog	0	42	93
Hog	75	51	98
Hog	150	43	100
Hog	300	36	90
Urea	50	39	88
Urea	100	42	85
Urea	200	43	88
Cattle	0	39	83
Cattle	120	35	80
Cattle	240	39	78
Cattle	480	34	65

• Microbial community structure

Additions of urea and hog and/or cattle manure affected the soil microbial community structure. For example, the FAME profiles of the whole soil microbial communities associated with the high rates of hog and cattle manure were significantly (PC1, $P<0.15$; PC2, $P<0.01$) different from the profiles of the communities associated with the control soils (Fig. 8). Similarly, the FAME profiles of the whole soil were altered due to additions of urea. The urea community was clearly separated from the communities associated with the hog and/or cattle manure treatments. However, no separation were observed using PCA amongst urea rates, thus

suggesting that a less diverse microbial community composition was present in the urea-treated soil (Fig. 8).

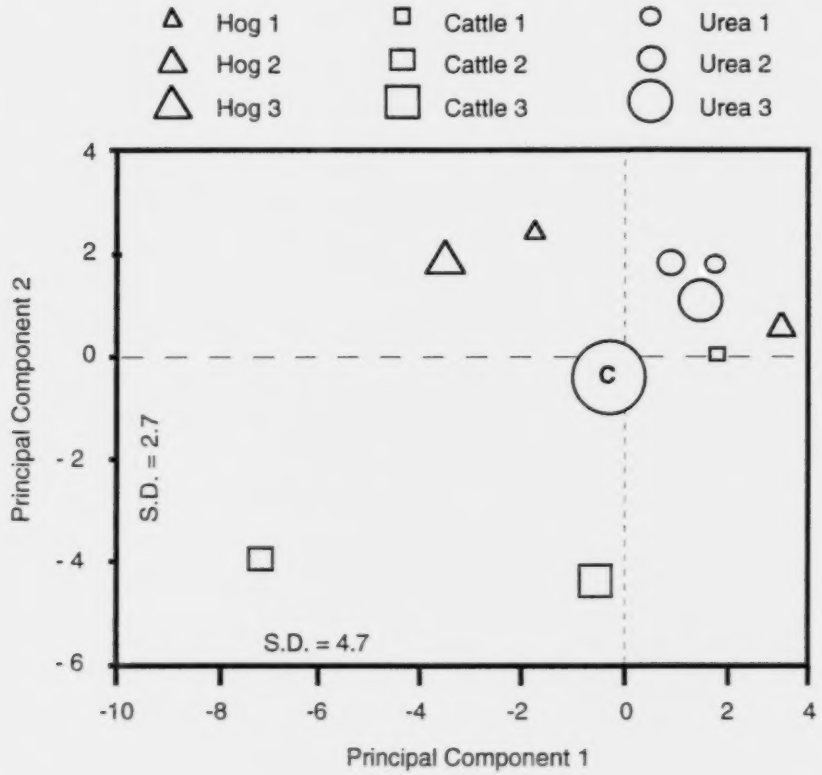

Figure 8. Principal component analysis (PCA) of FAME profiles of whole soil microbial community. Each symbol represents 4 soil samples at 42 days after application of hog manure, cattle manure, and urea at low, medium and high rates.

RESULTS AND DISCUSSION (YEAR III)

- **Soil Microbial Activity**

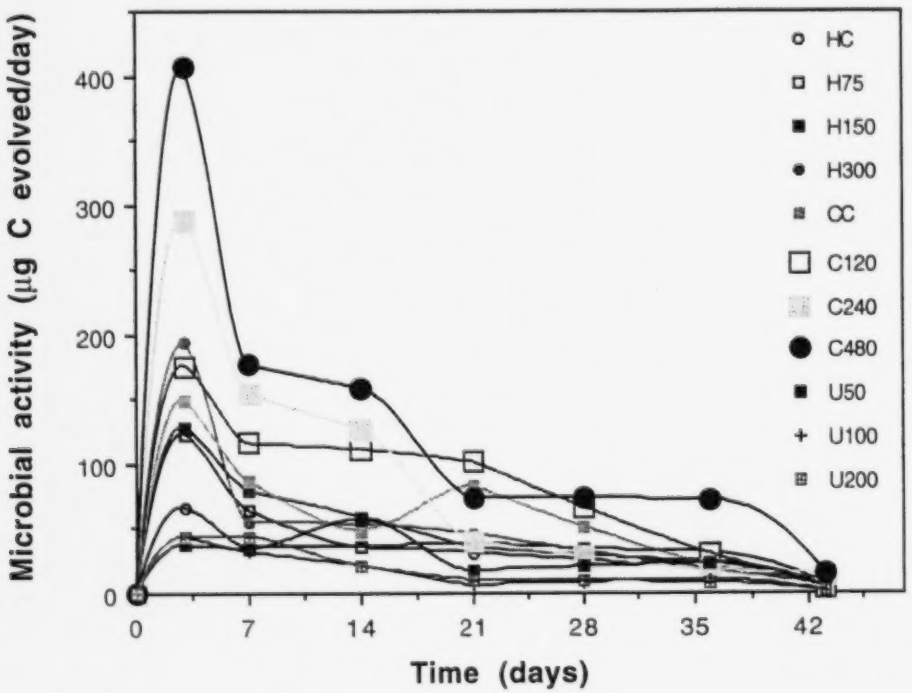

Figure 9. Effect of urea (U), hog (H) and cattle (C) manure application (kg N·ha^{-1}) on Dixon soil microbial activity (µg C·g^{-1} of soil) during the fall of 1999. Data are an average of 4 replicates assessed.

The results of microbial activity, assessed by CO_2 respiration on soil samples collected during the fall of 1999 after manure application were consistent with those obtained during the previous 2 years. These results indicate that applications of urea, hog and/or swine manure to the soil during the fall of 1999 affected the microbial activity differently. For example, during this 46-day soil incubation study, the microbial activity of soils treated with cattle manure was on average at least 2-fold higher than the soils injected with hog manure and/or *ca.* 3-fold higher than the soils treated with urea (Fig. 9). The higher microbial activity was pronounced at 3 days, then decreasing substantially, reaching values of 1.0 µg C·g^{-1} of soil at the end of the incubation period

which included treatment of soil with urea, hog and cattle manure. We have hypothesized from the previous two years data, that hog manure is more readily used as an energy source for microbial biomass and activity than cattle manure (Progress Report #1, submitted to ADF in August - 1998). The 1999 data supports this as similar to our previous observations, the lowest microbial activity was observed in the treatment receiving the high rate of urea (Fig. 9).

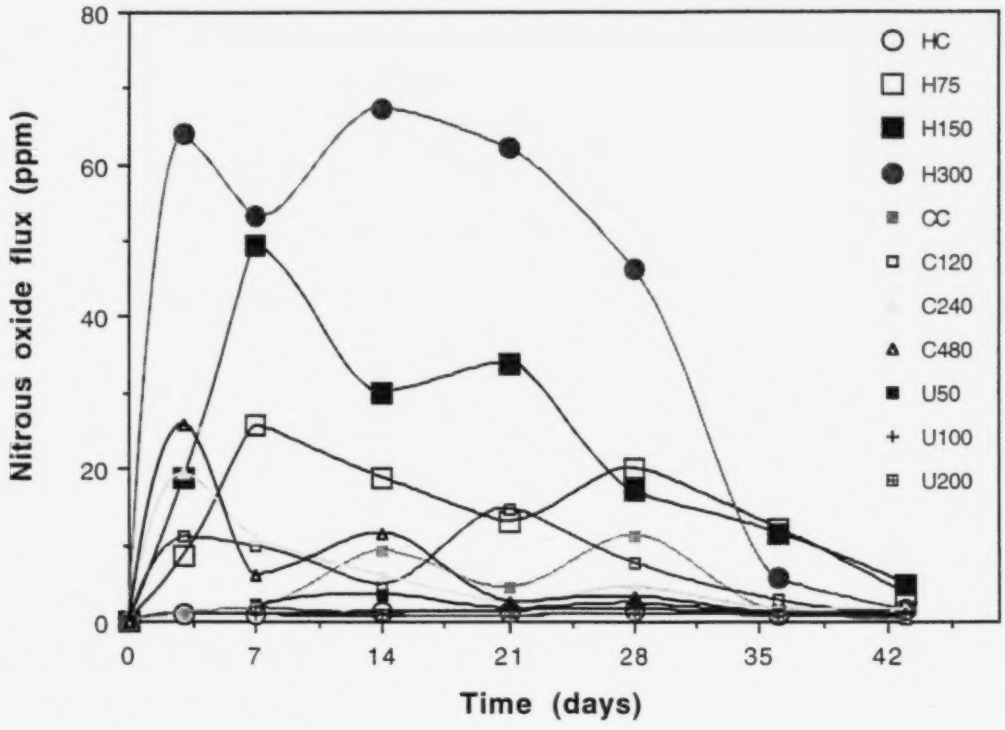

Figure 10. Effect of urea (U), hog (H) and cattle (C) manure application (kg $N \cdot ha^{-1}$) on nitrous oxide flux (ppm $N_2O \cdot 100\ g^{-1}$ of soil) at the Dixon site during the fall of 1999. Data are an average of 4 replicates assessed.

In 1999, new gas chromatography capabilities in our department also allowed us to measure nitrous oxide (N_2O) emissions as well as CO_2. Nitrous oxide emission rates increased successively up to 70.0 ppm during the first 14 days of incubation and fell thereafter reaching values of 0.3 ppm at the end of the study. Emission rates of nitrous oxide increased according to the levels of hog and cattle manure applications to the soil (Fig. 10). For example, addition of hog manure at 300 kg $N \cdot ha^{-1}$ produced the highest N_2O emission rate between 3 and 14 days during

the incubation study. Similarly, addition of cattle manure at 480 kg N·ha $^{-1}$ also produced the highest N_2O emission rates, but these rates were on average 2-fold lower that those corresponding to hog manure during the same period (Fig. 10). Our results also demonstrate that the total amount of nitrous oxide emission corresponding to the urea treatments seemed quite small compared with that of hog and cattle manure treatments.

- **Microbial populations**

Table 11. Culturable bacteria populations (cfu·g^{-1} soil) present in soil collected at the Dixon site during the fall of 1999 five days after manure application.

Treatment	Nitrogen (kg·ha^{-1})	Total heterotrophs (cfu·g^{-1} soil) ($\times 10^8$)	*Enterobacteriaceae* (cfu·g^{-1} soil) ($\times 10^2$)	Presumptive *Klebsiellae* (cfu·g^{-1} soil) ($\times 10^2$)
Hog	75	3.9	1.7	7.5
Hog	150	3.7	5.3	2.2
Hog	300	4.5	9.1	2.5
Urea	50	6.3	1.1	6.3
Urea	100	1.1	4.9	1.1
Urea	200	78.4	6.6	7.8
Cattle	120	77.2	15.1	4.4
Cattle	240	10.9	36.2	2.2
Cattle	480	6.5	75.6	0.0
Control	-	3.5	1.5	0.0

Results obtained during the fall 1999 sampling indicate that addition of cattle manure appeared to cause an increase in the number of total heterotrophs, whereas the addition of urea and/or hog manure promoted little change in the total heterotroph populations at the Dixon site (Table 11). However, the number of total heterotrophs in the soil were lower than in spring and summer of 1998, especially in the cattle manure plots. This may be related to the sampling time in 1999 (late fall) near freeze up when soil temperature is cold and timing of manure application relative to sampling. During the 1999 field study, *Enterobacteriaceae* were found in all soils treated with cattle and hog manure, urea as well as the untreated controls. It appears that these bacterial populations increased slightly according to the levels of manure and urea applications to the soil (Table 11). The cattle manure treated soils exhibited on average a 10-fold higher *Enterobacteriaceae* populations when compared to the remaining treatments, thus it is reasonable to

assume that cattle manure does contain slightly more *Enterobacteriaceae* as compared to hog manure (Table 11). Conversely, presumptive *Klebsiellae* were not detected in samples collected from the untreated controls, or from the cattle plot which received 480 kg N·ha^{-1}.

Table 12. Assessment of total bacterial populations (colony forming units - cfu) on soil samples collected at the Dixon experimental site during the summer of 2000.

Treatment	Rate (kg N·ha^{-1})	*Enterobacteriaceae* (× 10^4)	Presumptive *Klebsiellae* (× 10^5)	Total heterotrophs (× 10^7)
		(cfu per g dry soil)		
Hog	0	10.0	1.1	3.4
Hog	75	15.9	1.2	6.7
Hog	150	6.9	0.8	6.1
Hog	300	6.7	0.7	5.6
Urea	50	5.0	7.6	3.6
Urea	100	79.8	9.7	0.1
Urea	200	2.1	17.8	0.1
Cattle	0	22.0	2.8	3.7
Cattle	120	42.0	4.9	7.3
Cattle	240	122.0	0.1	0.2
Cattle	480	341.0	0.3	0.4

Samples taken from the plots in the summer of 2000 showed no consistent trends in numbers of *Klebsiella* as affected by cattle, hog or urea additions to the soil (Table 12). As in previous sampling periods, the *Enterobacteriaciae* numbers were elevated in plots receiving high rates of cattle manure.

• **Microbial identification: Fall of 1999**

Table 13. Microbial identification of bacterial strains isolated at the Dixon experimental site during the fall of 1999.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
16415	Hog	0	*Salmonella typhimurium*	0.370
16456	Hog	0	*Rahnella aquatilis*	0.492
16435	Hog	0	*Shigella flexneri*	0.123
16455	Hog	0	*Serratia marcescens*	0.451
16454	Hog	0	*Serratia marcescens*	0.256
16453	Hog	0	*Serratia marcescens*	0.418
16452	Hog	0	*Serratia marcescens*	0.353
16451	Hog	0	*Serratia marcescens*	0.363
16450	Hog	0	*Serratia marcescens*	0.342
16449	Hog	0	*Serratia marcescens*	0.505
16419	Hog	75	*Escherichia coli* subgroup A	0.668
16448	Hog	75	*Serratia marcenscens*	0.425
16436	Hog	75	*Pseudomonas stutzeri*	0.271
16447	Hog	75	*Serratia marcenscens*	0.408
16446	Hog	75	*Serratia marcenscens*	0.365
16416	Hog	75	*Acinetobacter baumannii*	0.519
16710	Hog	150	*Pseudomonas fluorescens*	0.837
16694	Hog	150	*Serratia marcenscens*	0.311
16427	Hog	150	*Serratia marcenscens*	0.443
16712	Hog	150	*Acinetobacter baumannii*	0.455
16417	Hog	150	*Acinetobacter baumannii*	0.377
16711	Hog	150	No Match	
16693	Hog	150	*Acinetobacter baumannii*	0.478
16428	Hog	300	*Pseudomonas mendocina*	0.734
16429	Hog	300	*Pseudomonas stutzeri*	0.754
16692	Hog	300	*Pseudomonas stutzeri*	0.633
16430	Hog	300	*Serratia marcenscens*	0.424
16691	Hog	300	*Acinetobacter baumannii*	0.573
16431	Hog	300	*Escherichia coli* subgroup B	0.659
16432	Hog	300	*Pseudomonas stutzeri*	0.544
16433	Hog	300	*Pseudomonas mendocina*	0.641
16434	Hog	300	*Pseudomonas stutzeri*	0.758
16418	Hog	300	*Yersinia enterocolitica*	0.611

Table 13. (cont'd) Microbial identification of bacterial strains isolated at the Dixon experimental site during the fall of 1999.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
16687	Urea	50	*Rahnella aquatilis*	0.519
16688	Urea	50	*Rahnella aquatilis*	0.591
16689	Urea	50	*Rahnella aquatilis*	0.592
16690	Urea	50	*Rahnella aquatilis*	0.623
16707	Urea	150	*Acinetobacter baumannii*	0.620
16708	Urea	150	*Enterobacter cloacae*	0.446
16709	Urea	150	*Acinetobacter baumannii*	0.452
16706	Urea	150	*Klebsiella pneumoniae*	0.534
16705	Urea	150	*Acinetobacter baumannii*	0.613
16704	Urea	150	*Enterobacter cloacae*	0.436
16703	Urea	150	*Enterobacter cloacae*	0.450
16702	Urea	150	*Enterobacter cloacae*	0.432
16701	Urea	150	*Enterobacter cloacae*	0.406
16445	Urea	150	*Escherichia coli* subgroup B	0.455
16684	Cattle	0	*Acinetobacter baumannii*	0.703
16437	Cattle	0	*Serratia marcescens*	0.273
16685	Cattle	0	*Serratia marcescens*	0.472
16438	Cattle	0	*Serratia marcescens*	0.331
16715	Cattle	0	*Pseudomonas putida*	0.875
16686	Cattle	0	*Acinetobacter baumannii*	0.665
16714	Cattle	0	*Pseudomonas fluorescens*	0.897
16713	Cattle	0	*Pseudomonas fluorescens*	0.898
16439	Cattle	0	*Serratia marcescens*	0.134
16695	Cattle	0	*Acinetobacter baumannii*	0.479
16421	Cattle	120	*Pseudomonas stutzeri*	0.596
16420	Cattle	120	*Pseudomonas stutzeri*	0.632
16696	Cattle	120	*Pseudomonas stutzeri*	0.757
16697	Cattle	120	*Acinetobacter baumannii*	0.308
16440	Cattle	120	*Serratia marcescens*	0.205
16441	Cattle	120	*Pseudomonas stutzeri*	0.727
16442	Cattle	120	No Match	
16458	Cattle	120	*Serratia marcescens*	0.336
16457	Cattle	120	*Serratia marcescens*	0.265
16426	Cattle	240	*Pseudomonas stutzeri*	0.460
16425	Cattle	240	*Pseudomonas stutzeri*	0.662
16424	Cattle	240	*Pseudomonas stutzeri*	0.458
16423	Cattle	240	*Pseudomonas stutzeri*	0.705
16444	Cattle	240	*Pseudomonas stutzeri*	0.606
16459	Cattle	240	*Serratia marcescens*	0.436
16443	Cattle	240	*Pseudomonas stutzeri*	0.573
16698	Cattle	240	*Pseudomonas stutzeri*	0.718

Table 13. (cont'd) Microbial identification of bacterial strains isolated at the Dixon experimental site during the fall of 1999.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
16460	Cattle	480	*Serratia marcescens*	0.377
16461	Cattle	480	*Serratia marcescens*	0.453
16699	Cattle	480	*Pseudomonas stutzeri*	0.821
16422	Cattle	480	*Pseudomonas stutzeri*	0.785
16700	Cattle	480	*Pseudomonas stutzeri*	0.798

Identification of soil bacteria isolated during the fall of 1999 and summer of 2000, indicated that Pseudomonads made up the greatest number of soil bacteria isolated from soils treated with urea, hog and cattle manure (Tables 13 & 14). For example, Pseudomonads comprised *ca.* 45% of the total bacteria processed during this period *i.e.*, of the 195 isolates identified, 88 isolates were *Pseudomonas* spp. Only 8 isolates which are listed as unidentified isolates have no match in the MIDI library thus, are unknown bacterial species. The remaining 99 bacterial isolates comprised of several *Enterobacteriaceae* including *Citrobacter*, *Rahnella*, *Salmonella*, *Serratia*, *Shigella*, *Yersinia* and *Escherichia coli*. *Enterobacteriaceae* were identified in all soil samples regardless to the treatment imposed (Tables 13 & 14). Similarly, other bacterial species identified included *Citrobacter* and *Yersinia* spp which may be isolated from substrates contaminated by feces. It is important to mention, however, that most of the bacterial species identified in this study occur naturally in soil, water and sewage and are classified as opportunistic human pathogens and/or of unknown clinical significance.

Table 14. Microbial identification of bacterial strains isolated at the Dixon experimental site during the summer of 2000.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
18036	Hog	0	*Pseudomonas fluorescens*	0.758
18035	Hog	0	*Pseudomonas fluorescens*	0.739
17681	Hog	0	*Pseudomonas putida*	0.794
18037	Hog	0	No Match	
18034	Hog	0	*Pseudomonas putida*	0.823
18029	Hog	0	No Match	
18044	Hog	0	*Pseudomonas putida*	0.184
18028	Hog	0	*Stenotrophomonas maltophilia*	0.682
18067	Hog	0	*Pseudomonas putida*	0.429

Table 14. (cont'd) Microbial identification of bacterial strains isolated at the Dixon experimental site during the summer of 2000.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
18027	Hog	0	*Pseudomonas fluorescens*	0.806
18026	Hog	75	*Pseudomonas fluorescens*	0.817
18025	Hog	75	*Citrobacter braakii*	0.539
18024	Hog	75	*Pseudomonas fluorescens*	0.828
18023	Hog	75	*Pseudomonas fluorescens*	0.844
18022	Hog	75	*Pseudomonas fluorescens*	0.845
18062	Hog	75	*Pseudomonas putida*	0.801
18021	Hog	75	*Pseudomonas putida*	0.740
18020	Hog	75	*Pseudomonas putida*	0.191
18018	Hog	75	*Pseudomonas putida*	0.632
18019	Hog	75	*Pseudomonas fluorescens*	0.785
18032	Hog	150	*Pseudomonas putida*	0.698
17706	Hog	150	*Stenotrophomonas maltophilia*	0.762
17705	Hog	150	*Pseudomonas fluorescens*	0.886
18031	Hog	150	*Stenotrophomonas maltophilia*	0.789
18059	Hog	150	*Stenotrophomonas maltophilia*	0.304
18066	Hog	150	*Pseudomonas putida*	0.529
17680	Hog	150	*Pseudomonas putida*	0.830
18061	Hog	150	*Pseudomonas putida*	0.427
18043	Hog	150	*Pseudomonas fluorescens*	0.736
18030	Hog	150	*Pseudomonas putida*	0.885
18016	Hog	300	*Pseudomonas putida*	0.840
18017	Hog	300	*Pseudomonas putida*	0.739
17694	Hog	300	*Stenotrophomonas maltophilia*	0.826
17693	Hog	300	*Pseudomonas fluorescens*	0.848
17664	Hog	300	*Pseudomonas putida*	0.795
17665	Hog	300	*Pseudomonas putida*	0.802
18033	Hog	300	*Pseudomonas putida*	0.866
18051	Hog	300	No Match	
17663	Hog	300	*Serratia liquefaciens*	0.317
17677	Hog	300	*Serratia liquefaciens*	0.487
17678	Hog	300	*Serratia liquefaciens*	0.351
17679	Hog	300	*Serratia liquefaciens*	0.506
18015	Urea	50	*Pseudomonas putida*	0.763
18056	Urea	50	*Oligella ureolytica*	0.242
18014	Urea	50	*Pseudomonas putida*	0.789
18013	Urea	50	*Pseudomonas putida*	0.801
18057	Urea	50	*Pseudomonas putida*	0.627
18058	Urea	50	*Pseudomonas fluorescens*	0.358
18011	Urea	50	*Pseudomonas fluorescens*	0.767
17666	Urea	50	*Pseudomonas putida*	0.833

Table 14. (cont'd) Microbial identification of bacterial strains isolated at the Dixon experimental site during the summer of 2000.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
18010	Urea	50	*Pseudomonas putida*	0.231
17722	Urea	50	*Pseudomonas putida*	0.750
18012	Urea	100	*Pseudomonas putida*	0.698
18065	Urea	100	*Pseudomonas putida*	0.780
17721	Urea	100	*Pseudomonas fluorescens*	0.833
18055	Urea	100	*Pseudomonas putida*	0.799
17720	Urea	100	*Pseudomonas putida*	0.842
17667	Urea	100	*Rahnella aquatilis*	0.357
18063	Urea	100	*Pseudomonas fluorescens*	0.542
17719	Urea	100	*Pseudomonas putida*	0.828
18064	Urea	100	*Pseudomonas fluorescens*	0.537
17670	Urea	100	*Pseudomonas fluorescens*	0.744
18053	Urea	100	*Pseudomonas fluorescens*	0.658
18052	Urea	100	*Pseudomonas fluorescens*	0.631
18049	Urea	200	*Pseudomonas fluorescens*	0.156
17671	Urea	200	*Rahnella aquatilis*	0.649
17682	Urea	200	*Pseudomonas fluorescens*	0.764
17686	Urea	200	*Pseudomonas fluorescens*	0.932
17685	Urea	200	*Pseudomonas putida*	0.810
18050	Urea	200	*Pseudomonas fluorescens*	0.740
17684	Urea	200	*Pseudomonas fluorescens*	0.822
17683	Urea	200	*Pseudomonas fluorescens*	0.819
17687	Urea	200	*Pseudomonas putida*	0.538
17695	Cattle	0	*Pseudomonas fluorescens*	0.848
17669	Cattle	0	*Serratia liquefaciens*	0.423
17668	Cattle	0	*Pseudomonas putida*	0.855
17672	Cattle	0	*Pseudomonas putida*	0.865
17699	Cattle	0	*Pseudomonas fluorescens*	0.922
18038	Cattle	0	No Match	
17696	Cattle	0	*Salmonella typhimurium*	0.143
17697	Cattle	0	*Yersinia enterocolitica*	0.481
17698	Cattle	0	No Match	
17700	Cattle	0	*Yersinia enterocolitica*	0.560
17701	Cattle	0	No Match	
18048	Cattle	120	*Stenotrophomonas maltophilia*	0.624
17673	Cattle	120	*Pseudomonas putida*	0.682
17702	Cattle	120	*Pseudomonas fluorescens*	0.779
17703	Cattle	120	*Stenotrophomonas maltophilia*	0.760
18060	Cattle	120	*Stenotrophomonas maltophilia*	0.634
18047	Cattle	120	*Pseudomonas fluorescens*	0.790
18045	Cattle	120	No Match	
18054	Cattle	120	*Pseudomonas putida*	0.791

Table 14. (cont'd) Microbial identification of bacterial strains isolated at the Dixon experimental site during the summer of 2000.

Sample	Treatment	Rate (kg N·ha^{-1})	MIDI - Identification	Similarity Index
18046	Cattle	120	*Pseudomonas fluorescens*	0.779
17674	Cattle	120	*Rahnella aquatilis*	0.425
17704	Cattle	120	*Pseudomonas putida*	0.684
17688	Cattle	240	*Pseudomonas putida*	0.719
17689	Cattle	240	*Pseudomonas putida*	0.903
17690	Cattle	240	*Pseudomonas fluorescens*	0.830
17675	Cattle	240	*Pseudomonas putida*	0.760
17691	Cattle	240	*Pseudomonas fluorescens*	0.953
17692	Cattle	240	*Pseudomonas fluorescens*	0.824
17707	Cattle	240	*Pseudomonas fluorescens*	0.899
17718	Cattle	240	*Pseudomonas fluorescens*	0.765
17717	Cattle	240	*Pseudomonas putida*	0.769
17716	Cattle	240	*Pseudomonas fluorescens*	0.795
17715	Cattle	240	*Pseudomonas putida*	0.754
17713	Cattle	480	*Pseudomonas putida*	0.764
17712	Cattle	480	*Pseudomonas putida*	0.896
18039	Cattle	480	*Stenotrophomonas maltophilia*	0.532
17711	Cattle	480	*Pseudomonas fluorescens*	0.924
17710	Cattle	480	*Pseudomonas putida*	0.900
17709	Cattle	480	*Pseudomonas putida*	0.573
18040	Cattle	480	*Pseudomonas putida*	0.638
17676	Cattle	480	*Serratia liquefaciens*	0.851
18041	Cattle	480	*Pseudomonas fluorescens*	0.524
18042	Cattle	480	*Pseudomonas putida*	0.625
17708	Cattle	480	*Stenotrophomonas maltophilia*	0.871

- **Incidence of soil entero-pathogens (fecal coliforms)**

Table 15. Presumptive and confirmatory tests of fecal coliforms in soils from the Dixon site sampled in the fall of 1999 five days after manure applications.

Treatment	Nitrogen ($kg \cdot ha^{-1}$)	Most Probable Number	
		Presumptive ($\times 10^2$)	Confirmatory ($\times 10^2$)
		..... (counts per 100 ml)	
Hog	0	2.0	ND*
Hog	75	2.0	ND
Hog	150	2.0	ND
Hog	300	2.0	ND
Urea	50	ND	ND
Urea	100	ND	ND
Urea	200	1.0	ND
Cattle	0	ND	ND
Cattle	120	8.5	ND
Cattle	240	19.0	15.0
Cattle	480	6.0	2.0

ND* not detected.

Presumptive and confirmatory tests of fecal coliforms in soils from the Dixon site sampled in the fall of 1999 indicated that fecal coliforms were isolated in soils which received hog or cattle manure five days after manure applications. However, our test also indicated that fecal coliforms were only confirmed in soils which received high rates of cattle manure *i.e.*, over 240 $kg \cdot ha^{-1}$ (Table 15). No fecal coliforms were found in soil receiving urea (Table 15).

Table 16. Presumptive and confirmatory tests of fecal coliforms in soils from the Dixon site sampled during the summer of 2000.

Treatment	Nitrogen ($kg \cdot ha^{-1}$)	Most Probable Number	
		Presumptive ($\times 10^2$)	Confirmatory ($\times 10^2$)
		..... (counts per 100 ml)	
Hog	0	0.8	ND*
Hog	75	4.1	ND
Hog	150	1.5	ND
Hog	300	14.0	ND
Urea	50	1.9	ND
Urea	150	11.0	1.2
Urea	300	0.3	ND
Cattle	0	0.6	ND
Cattle	120	2.3	ND
Cattle	240	48.0	0.08
Cattle	480	210.0	ND

ND* not detected.

Similar to the results obtained during the fall of 1999, presumptive and confirmatory tests of fecal coliforms in the soil indicated that fecal coliforms were isolated in soils which received hog or cattle manure in the summer of 2000. However, our test also indicated that fecal coliforms were isolated and confirmed in soils receiving urea and/or 240 $kg \cdot ha^{-1}$ of cattle manure (Table 16). The fact that fecal coliforms were confirmed in the urea-treated plots may be associated with cross contamination during manuring operations associated with numerous manure applications and close proximity of the plots at the experimental site. No fecal coliforms were confirmed in soils receiving hog manure (Table 16).

- **Foliar disease assessment**

A plant disease-survey was performed at the Dixon field site during the summer of 2000 to assess disease incidence and severity in the canola, as was done for the 1999 barley crop. Plants were characterized for the presence of plant disease. Results from the foliar disease assessment indicate that addition of urea, hog or cattle manure to the soil did not cause visual foliar disease in canola during the 2000 crop season. However, with the exception of the controls from the hog manure plots, all treatments contained traces to 1-5% of Aster yellows. The reason our untreated control plots exhibited a lower Aster yellows incidence may be related to the lower height of plants

in the control plots due to stress from lack of nutrients, especially N. We did not find traces of diseases caused by *Alternaria* and *Sclerotinia* species in any of the plants.

• Microbial community structure

In contrast to the previous year, additions of urea and hog and/or cattle manure during the fall of 1999 following manure application did not show large differences in the soil microbial community structure. Although there were separations on the FAME profiles of the whole soil microbial communities, they were not significantly (PC1, $P<0.58$; PC2, $P<0.72$) different from the profiles of the communities associated with the control soils (Fig. 11). However, there was a trend for separation as the FAME profiles of the whole soil were altered due to additions of cattle and/or hog manure. The microbial community associated with the highest cattle manure rate was clearly separated from the communities associated with the whole soil and/or urea treatments. However, no separation was observed using PCA amongst urea and/or hog manure rates. These results may be explained by the fact that the soil was sampled soon after manure applications during the fall, when the soil was cold and with insufficient time for the microbial populations to respond to the new source of substrate (Fig. 11).

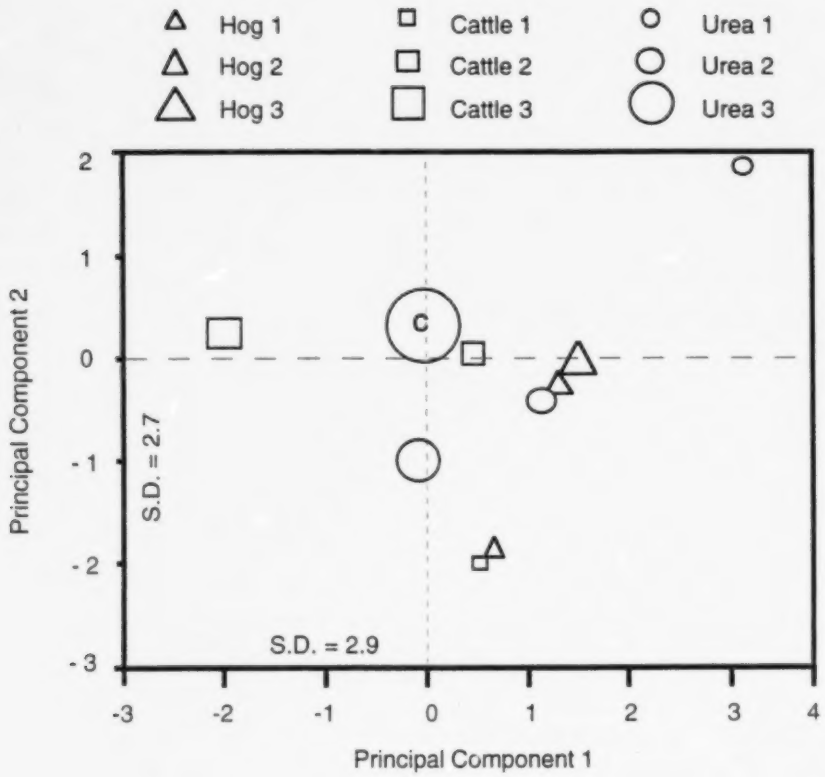

Figure 11. Principal component analysis (PCA) of FAME profiles of whole soil microbial community during the Fall 99. Each symbol represents 4 soil samples at 5 days after application of hog manure, cattle manure, and urea at low, medium and high rates.

Similar to the results obtained in the previous year for the in - growing season (summer) sampling, additions of urea and hog and/or cattle manure affected the soil microbial community structure (Fig. 12). The results obtained in the summer of 2000 confirm that the FAME profiles of the whole soil microbial communities associated with the medium and high rates of hog and cattle manure were significantly (PC1, $P<0.005$; PC2, $P<0.09$) different from the profiles of the communities associated with the control soils (Fig. 12). Similarly, the FAME profiles of the whole soil were altered due to additions of urea. The urea community was clearly separated from the communities associated with the hog and/or cattle manure treatments. However, no separation were observed using PCA amongst urea and/or cattle manures rates, thus confirming that a less diverse microbial community composition is present in the urea-treated soil (Fig. 12).

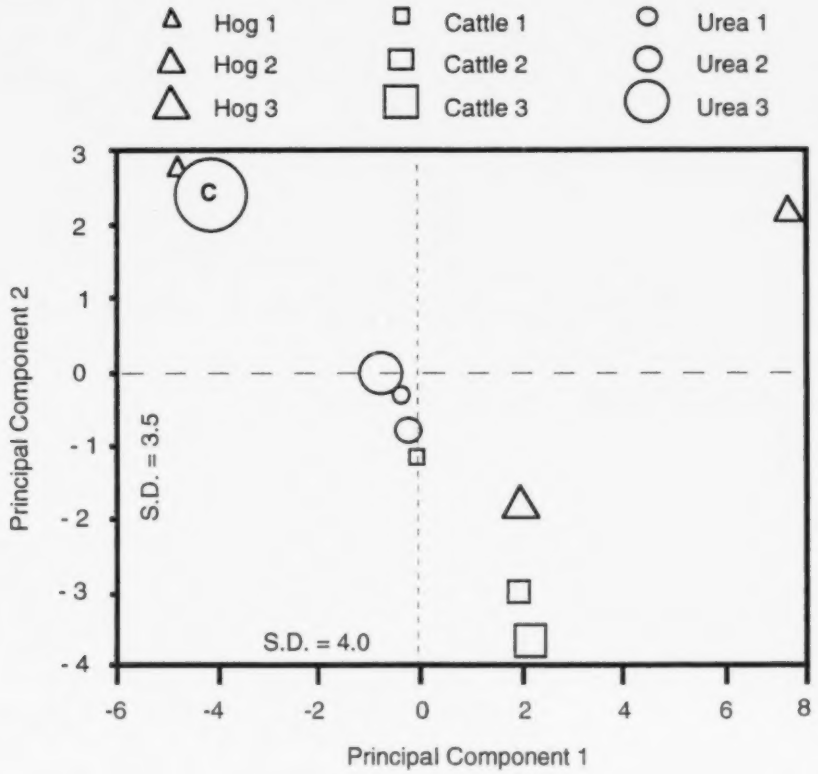

Figure 12. Principal component analysis (PCA) of FAME profiles of whole soil microbial community during the summer of 2000. Each symbol represents 4 soil samples at 45 days after application of hog manure, cattle manure, and urea at low, medium and high rates.

CONCLUSIONS

Results from this three-year study demonstrated that organic matter in liquid hog effluent was more rapidly decomposed than cattle manure. Hog manure was more readily used as a substrate for energy, nutrients and biomass production by the soil microbial population than cattle manure. Several potential pathogenic bacteria were isolated and identified from manure-treated soils. Some isolates were ubiquitous to soil and fresh water and are of unknown clinical significance. Some species are potential pathogenic bacteria known to invade human urinary tract and cause lesions on human skin. Other isolates occur in manures, soil and polluted water and are occasionally opportunistic pathogens to humans and animals. We found no evidence that addition of hog manure increased the incidence of pathogenic microbial isolates. These results provide new

information on microbial activity, populations and communities in manure amended soil. However, the link between incidence of microorganisms in soil and potential health hazards is complex and cannot be derived or postulated simply from their incidence and activity in the soil. Application of hog or cattle manure did not cause increases in visually detectable foliar diseases in wheat. Leaf spot incidence was lower and leaves were bigger and more vigorous in plants from manure or urea treated soil. Although application of hog manure, cattle manure and urea increased root rot incidence in wheat in 1998, this did not appear to adversely affect plant growth. In fact, our results demonstrated that soil application of hog and/or cattle manure improved crop vigor which may have led to less impact of common root rot disease on crop yield.

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Deliverables and performance indicators

We have achieved the milestones indicated in our research proposal as listed below.

Mile-stones	Description of activities and deliverables	Start and completion	Status
1	Isolation of microbial community from soils already collected from the manure plots in Saskatchewan.	Feb. 1998-Mar. 1998	Completed
2	Identification and monitoring of microbial communities in soils collected from field plots using FAME profiles (HP/MIDI System).	Apr. 1998-June 1998	Completed
3	Plant disease assessment, field sampling and isolation of microbial community from the manure plots.	June 1998-Oct. 1998	Completed
4	Assessment of human pathogenic bacteria in soils and crops under manure applications.	Oct. 1998-Nov. 1998	Completed
5	Isolation of microbial community from soils collected from the manure fields under crop in the second year study.	Feb. 1999-June 1999	Completed
6	Plant disease assessment, field sampling and isolation of microbial community from the manure plots in the second year.	June 1999	Completed
7	Assessment of human pathogenic bacteria on soils and crops under manure applications in the second year study.	Jan. 2000	Completed
8	Isolation of microbial community from soils collected from the manure fields under crop in the third year study.	Feb. 2000-June 2000	Completed
9	Plant disease assessment, field sampling and isolation of microbial community from the manure plots in the third year.	June 2000	Completed
10	Management strategies to conserve and promote beneficial soil microbial communities in agro-ecosystems subjected to manure applications.	Apr. 2001	Completed
11	Final Report.	June 2001	Completed

d) Personnel

(*i*) List of participants in the project:

Dr. J. Schoenau (Principal applicant) - University of Saskatchewan;
Dr. J. R. de Freitas (Co-applicant) - University of Saskatchewan;
Dr. S. Boyetchko (Co-applicant) - Agriculture and Agri-Food Canada - Saskatoon;
Sonia Blechinger (Assistant Research Technician).

e) Equipment:

None to report.

f) Project developed materials:

None to report.

g) Project photos:

None to report.

h) Acknowledgment:

Acknowledgments are made to ADF at the end of the following project public information materials.

i) Project Public Information Materials:

The following papers were given at Local and/or International Conferences on Soils and Agronomy:

Title: "**EFFECT OF MANURE APPLICATIONS ON SOIL MICROBIAL ACTIVITY**" by: De Freitas, J.R.; Schoenau; Boyetchko, S.M.; & Blechinger, S.B. 1998. Proceedings of Soils and Crops '99. Extension Division, University of Saskatchewan, Saskatoon - SK. Feb. 25-26, 1999. pp. 489-500.

Title: "**MICROBIAL ACTIVITY AND INCIDENCE OF SOIL BORNE PLANT AND HUMAN PATHOGENS ON SOIL TREATED WITH MANURE**", by: De Freitas, J.R.; Schoenau, J.J.; Boyetchko, S.M. and Blechinger, S.A., was presented at the **Manure Management "99 Conference**. Proceedings of a Tri-Provincial Conference on Manure Management. University of Saskatchewan, Extension Division, Saskatoon - SK. June 22-25, 1999, Saskatoon, SK. pp. 565-579.

Title: "**THE IMPLICATION OF MANURE USE ON MICROBIAL ACTIVITY AND PATHOGEN INCIDENCE IN SASKATCHEWAN SOILS**", by: De Freitas, J.R.; Schoenau, J.J.; Boyetchko, S.M. and Blechinger, S.A., was presented at the 90th Annual Meeting of the American Society of Agronomy - ASA, CSSA, SSSA, Salt Lake City, Utah, USA. Oct. 31 - Nov. 4, 1999.

Title: "**THE IMPLICATION OF MANURE USE ON MICROBIAL ACTIVITY AND PATHOGEN INCIDENCE IN SASKATCHEWAN SOILS**", by: De Freitas, J.R.; Schoenau, J.J.; Boyetchko, S.M. and Blechinger, S.A., was presented at the **Soils and Crops 2000**. Saskatoon - SK. Feb. 24 - 25, 2000.

Title: "**THE USE OF HOG MANURE: IMPLICATIONS REGARDING HUMAN AND CROP HEALTH**", by: De Freitas, J.R. & Schoenau, J.J., was be presented at the **Sask. Ag. and**